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Cytological Studies in the Genus Gaura

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With twenty-five Figures in the Text

INTRODUCTION

ACCORDING to Engler's (1898) system of classification, the genus Gaura belongs to the tribe Gaureae of the family Onagraceae. The genus consists of approximately twenty species, distributed in Mexico and temperate North America. *G. biennis* and *G. Lindheimeri* are the two introduced garden plants. Although opinion varies regarding the relationship of this genus to other genera of the family Onagraceae, the marked morphological similarities between Gaura and Oenothera are well recognized.

It is now well established that critical cytological information is of great help in the elucidation of the relationships between different groups of plants. The study of the relation of chromosomes to nucleoli has not only confirmed the above view but has been profitably used in recent years for such purposes (Gates, 1937; Bhaduri, 1940; Pathak, 1940, 1940a, and 1940b; and Sikka, 1940, 1940a). Considering the vast amount of cytological and genetical work done in the genus Oenothera and the very important results obtained, it is surprising to find that very little cytological information, besides the count of haploid and diploid chromosome numbers, is available for the other genera of the family Onagraceae. While studying the cytology of the genus Oenothera (cf. Bhaduri, 1940) it was realized that in order to get a clear picture of the steps of nuclear evolution in this genus observations on similar lines are essential in related genera. The present paper is the first critical cytological account in the genus Gaura. Observations in the present paper have been restricted chiefly to the study of the relation of chromosomes to nucleoli, but the account is as comprehensive as the material and time permitted.

MATERIAL AND METHODS

Material for the present study was obtained from a single plant of *Gaura Lindheimeri*, grown in the University Botanical Garden, Bristol, during October 1939. Root-tips were collected later from germinating seeds, grown in equal proportions of sand and loam in the laboratory, the seeds being obtained from Kew. Flower-buds were fixed in medium Flemming, with pretreatment in Carnoy's fluid. Root-tips were fixed in Levitsky's chromic

formalin mixture in the proportion of 1 : 1. For flower-bud material, paraffin sections, 20 μ to 22 μ thick, were cut and stained by Newton's gentian violet iodine method as modified by La Cour. For root-tip material, paraffin sections 12 μ thick were cut and stained either with gentian violet or with Feulgen light green method (Semmens and Bhaduri, 1940).

The observations and drawings were made using a Zeiss 2 mm. apochromatic objective 1·4 N.A. with an immersion aplanatic condenser 1·3 N.A., and compensating eyepieces × 30 and × 20.

OBSERVATIONS

G. Lindheimeri has $2n = 14$ chromosomes (Figs. 1, 2). This observation is in agreement with that of Johansen (1929), who found the same number in both *G. Lindheimeri* and *G. coccinea*. The somatic chromosomes of Gaura are very short, even shorter than in Oenothera. While the length of the metaphase chromosomes of Oenothera vary from 3·8 μ to 2·5 μ (Bhaduri, 1940), in Gaura they are 2·8 μ to 1·9 μ. In spite of the shortness of the chromosomes, three different sizes of chromosomes, long, medium, and short, with median or slightly sub-median primary constrictions, have been observed. Due to gradual gradation of sizes, however, it becomes difficult to make the intermediate chromosomes a distinct group. The fourteen chromosomes of this species can be grouped as follows: 2 pairs of long chromosomes with median constrictions (*Lm*), 2 pairs of intermediate-sized chromosomes with median constrictions (*Im*), and 1 pair of short chromosomes with median constrictions (*Sm*). The remaining four chromosomes are distinguished from the rest by having a marked secondary constriction in each. Two of these four chromosomes have sub-median primary constrictions (*Stsm*) and the other two median primary constrictions (*Stm*) (Figs. 1, 2, 3). The secondary constrictions are well marked in Gaura as in Oenothera (Bhaduri, 1940). They are much more conspicuous than the primary constrictions. The appendage separated from one of the arms of the chromosome by a secondary constriction is very big (sometimes as big as the short arm of a chromosome) and is not morphologically comparable to the satellites of other plants. The secondary constriction in one of the chromosomes with sub-median primary constriction is extraordinarily big, and this seems to be a constant character (Fig. 3). Similar observations have also been made previously (Bhaduri, 1940) in *Oenothera augustissima* var. *quebecensis*. It cannot be said at present whether this chromosome produces the largest of the four nucleoli observed in the somatic nuclei of this plant. It appears, however, that this chromosome and the other one with sub-median primary constriction form a heteromorphic pair (Fig. 3). The fine thread representing the secondary constriction is Feulgen positive.

The maximum number of nucleoli in the somatic nuclei has been found to be four (Fig. 4), corresponding to the four chromosomes with secondary

constrictions. During prophase four of the fourteen chromosomes have been found remaining attached to the fused nucleolus (Fig. 5). Due to the extreme smallness of the chromosomes the origin of the four nucleoli from the four secondary constrictions could not be traced; the same can, however, be
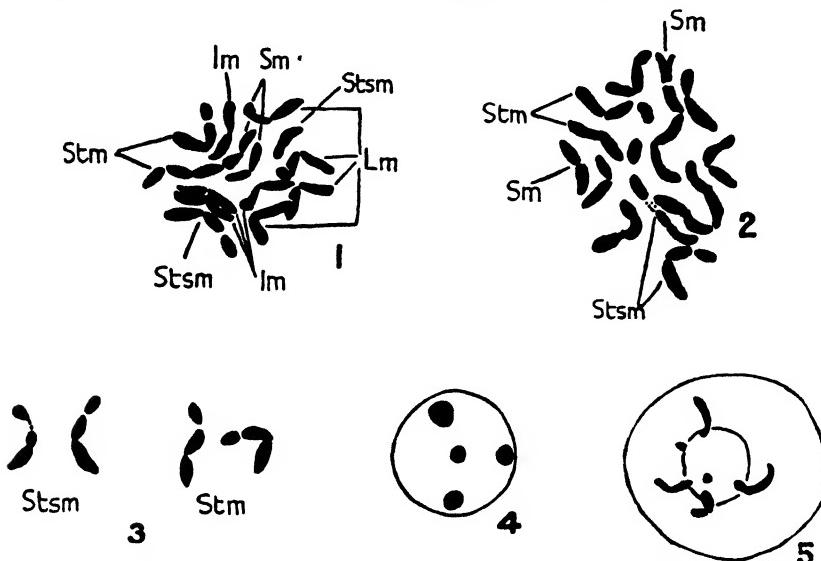
FIGS. 1–5. Figs. 1–2. Somatic metaphase plates of *G. Lindheimeri*. *Lm, Im,* and *Sm* representing respectively, long, intermediate, and small chromosomes with median constrictions. *Stm* and *Stsm*, representing chromosomes with secondary constrictions with either median or submedian primary constrictions respectively. Fig. 3. Two pairs of chromosomes with secondary constrictions drawn separately. Fig. 4. Four nucleoli of three different sizes. Fig. 5. Attachment of four chromosomes to the nucleolus by their secondary constrictions. × 3,500.

safely inferred from the nature of the attachment of the four chromosomes to the prophase nucleolus. The secondary constrictions are therefore nucleolar constrictions, and these chromosomes with secondary constrictions are homologous to the Sat-chromosomes of other plants. The nucleoli are of three different sizes, one big, one small, and two intermediates (Fig. 4). The maximum number of nucleoli could only be ascertained with accuracy from preparations stained with Feulgen and light green. Preparations stained with gentian violet were found to be quite unreliable for this purpose because it is very difficult to distinguish between the chromocentres and the small nucleoli during early stages, both being stained similarly.

The resting nuclei of the somatic cells are characterized by the presence of a definite number of small, deeply stained bodies. They are Feulgen positive and also stain deeply with acetocarmine. Their number corresponds to the diploid number, $2n = 14$, and they represent true chromocentres, as also observed in Oenothera (Bhaduri, 1940).

The sporogenous tissue of the anther consists of a string of cells which are

more or less rectangular during early stages of meiosis. They round off gradually along with the progress of the meiotic prophase and become almost spherical by the time the nuclei arrive at the diakinesis stage. During the course of this change in shape of the pollen mother cell a marked change in their outer
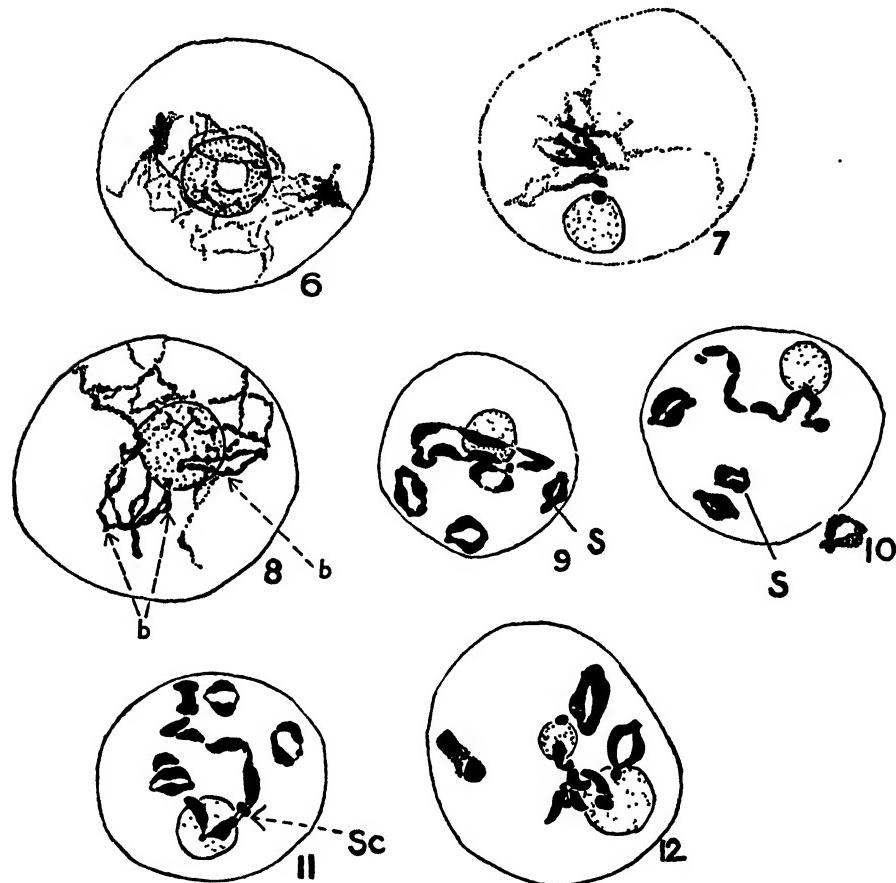
FIGS. 6–12. Figs. 6–7. Zygotene stage. The nucleolus with a typical nucleolar body in Fig. 7. Fig. 8. Pachytene; *b*, representing the bivalent pairs. Fig. 9. Showing maximum catenation, $(6)+4_{11}$. Note the close association of the (6) and a bivalent to the nucleolus. S, representing the smallest bivalent. Fig. 10. A chain of 6 chromosomes attached to the nucleolus. One bivalent displaced by the microtome knife. Fig. 11. Broken (6) attached to the nucleolus. *Sc*, secondary constriction. Fig. 12. Two nucleoli of unequal sizes. One chromosome clearly attached to the smaller nucleolus by a secondary constriction. × 2,400.

wall could be noticed. The originally thin outer wall becomes markedly thickened and appears to become mucilaginous. This wall holds the gentian violet stain feebly and appears greyish in the final preparation. Due to their very delicate nature and weak staining, the continuity of the zygotene threads could not be traced satisfactorily to give any evidence as to the nature of

pairing of chromosomes. The threads generally form a contracted mass and do not stain brightly everywhere (Fig. 6). One sees deeply stained patches here and there (Fig. 7). It is not safe, however, to conclude from this evidence that the deeply stained portions are heterochromatic in comparison to the very feebly stained euchromatic portions. The pachytene threads are more clearly defined and a considerable length of some of these threads could be traced at places. Parasynaptic association of portions of such chromatids were clearly indicated in places. Indication of interstitial chiasma formation between such chromatids could be seen in Fig. 8. In the same figure it will be noticed that a pair of chromatids are held by terminal chiasmata. These two latter pairs of chromatids represent two of the four ring bivalent pairs observed during diakinesis.

A distinct catenation of chromosomes during diakinesis has been observed in this species, the maximum catenation being a ring of six and four bivalents (Fig. 9). Instead of a ring of six, not infrequently, a chain of six chromosomes was observed (Fig. 10). Of the four bivalent pairs, one pair appears to be smaller than the rest and probably represents the smallest pair of chromosomes of the somatic complements (Figs. 9, 10).

Generally the pollen mother cells show the presence of only one big nucleolus (Figs. 9, 10, 11). This nucleolus is the fusion product of four nucleoli observed in somatic nuclei. In few instances, after very careful search, one big and one small nucleolus could be observed (Fig. 12). The ring of six contains a pair of chromosomes with secondary constrictions, and in every case where the ring could be distinguished they were found attached to the nucleolus (Figs. 9, 10, 11). One bivalent, not the smallest one, which was also found attached to the nucleolus consists of the other two chromosomes with secondary constrictions (Fig. 9). Although the secondary constrictions of chromosomes could not always be identified during this stage, clear cases showing their presence and attachments to the nucleoli at those points have been clearly observed (Figs. 11, 12).

During metaphase the ring of six chromosomes arranges itself at the equatorial region of the spindle in the characteristic zigzag manner, so that alternate chromosomes in the ring can go to opposite poles (Figs. 13, 14, 15). The separation of the four bivalents during first anaphase is not uniform. One pair seems to separate ahead of the rest. The smallest pair generally separate last (Figs. 13, 14, 16, 17). Sometimes the separation of a bivalent seems to be incomplete. The unseparated segments remain attached to the already free segments by means of weakly stained portions (Figs. 13–18). Similar observations have also been made by a number of investigators in Oenothera (Catcheside, 1932; Bhaduri, 1940; Pathak, 1940; and Sikka, 1940). According to Catcheside, this appearance is brought about by incomplete terminalization of the chiasmata. It appears, however, that this microscopical appearance can be brought about by other means and be interpreted differently. The presence of the weakly stained portions, separating these

segments from the body of the chromosomes, leads to the view that the matrix substance has something to do with these appearances, and the explanation of failure of terminalization of chiasmata only does not give a clear picture of the situation. According to Marquardt (1937) these connecting
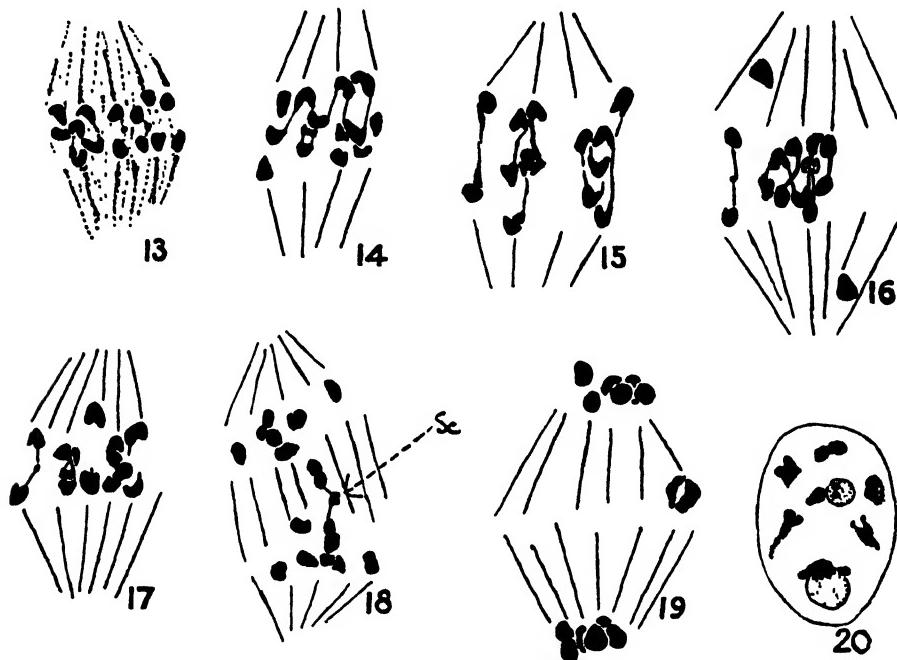
FIGS. 13–20. Fig. 13. Anaphase I. Note the early separation of two bivalents and the connecting bridges in the other two. Fig. 14. Zigzag orientation of (6). Fig. 15. Same; note the conspicuous connecting bridge in each bivalent. Figs. 16–17. Early separation of one bivalent. Fig. 18. Note the delay in separation of one pair. *Sc*, secondary constriction. Fig. 19. One bivalent failed to separate. Fig. 20. An interkinetic nucleus showing the origin of the nucleoli. × 2,400.

'bridges' represent the agglutination of the matrix and have nothing to do with chiasmata. In Fig. 18 this appearance can be explained as due to the delay in separation of the appendages separated by secondary constrictions. Then again, the presence of small bodies, the terminal granules, as observed in both meiotic and mitotic chromosomes in Oenothera (Bhaduri, 1940; Sikka, 1940; and Pathak, 1940), in association with the matrix substance of the chromatids may also be responsible for these appearances. Complete failure of separation of a bivalent has been observed in few instances (Fig. 19).

During interkinesis the development of two nucleoli and their attachment to two different chromosomes by secondary constrictions in a nucleus (Fig. 20) shows conclusively that the four chromosomes with secondary constrictions constitute two pairs which disjoin regularly during first anaphase. This is

further evidenced by the fact that from interkinesis to fully formed pollen grain stages, the presence of more than two nucleoli in a nucleus has never been observed. Regarding the size relation of nucleoli in the above stages it
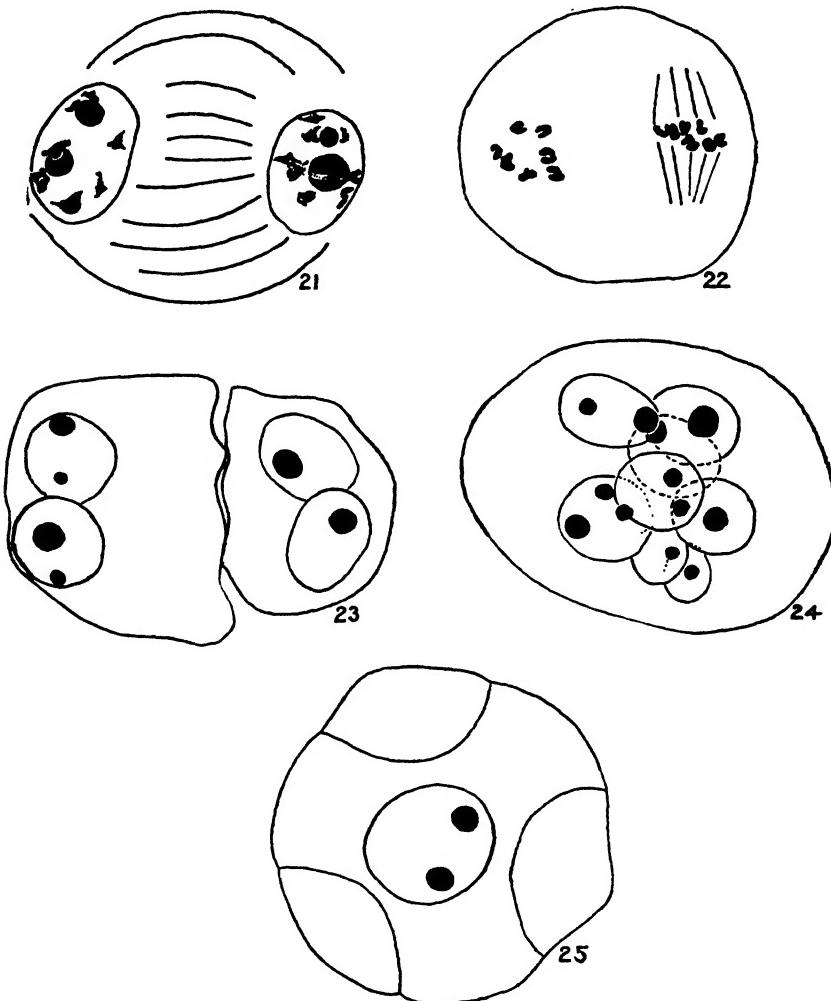
Figs. 21-5. Fig. 21. Interkinesis; one nucleus with the big and small and the other nucleus with two intermediate nucleoli. Fig. 22. Normal second division. Fig. 23. Incomplete furrowing. Fig. 24. A giant pollen grain with eight nuclei. One nucleus has three nucleoli. Fig. 25. A pollen grain with two nucleoli of equal sizes. × 2,400. Figs. 21-5. Reduced to two-thirds of the original drawings.

has been noted that in the majority of cases there are two nucleoli of unequal sizes (Figs. 20, 21). In few instances, however, two nucleoli of the same size have been observed (Figs. 21, 25).

The distribution of the chromosomes during first and second divisions, on the whole, seems to be regular (Fig. 22), no indication of secondary association

of chromosomes being noticed. The general condition seems to be one in the middle surrounded by six others, as theoretically expected for seven chromosomes on the 'floating magnet' conception.

Cytokinesis takes place by furrowing followed by cell plate formation, as generally observed in dicotyledonous plants. Well-developed pollen grains remain for a considerable period within the pollen mother cell, protected by a thick pellicle of mucilaginous wall.

Various abnormalities during tetrad formation have been observed in the present material. A general condition is the complete failure of furrowing and cell plate formation, the whole pollen mother cell appearing as a huge pollen grain with four free nuclei, each nucleus containing two nucleoli. These grains sometimes have one to three nuclei instead of four. It cannot be said with certainty whether these latter conditions are due to fusion or degeneration of some of the nuclei. Division of the pollen mother cell into two cells, each having one, two, or more nuclei, has sometimes been observed. Incomplete furrowing was also noted in a few instances (Fig. 23). In a number of such giant pollen grains, 5, 6, or 8 nuclei instead of 4 have been observed (Fig. 24). Sometimes one or two of these nuclei appear as micronuclei. Although no extra divisions of the nuclei or extra spindle figures have been noted which could have explained the formation of more than four nuclei, the presence of more than eight nucleoli in a pollen mother cell indicates extra divisions. Rare cases, showing the presence of three nucleoli in a nucleus of such a giant grain, indicate further irregular separation or extra divisions of chromosomes (Fig. 24). These giant pollen grains or metamorphosed pollen mother cells show the same kind of thickening and lobing of the outer wall as found in a normal pollen grain. Regarding the viability and fate of these abnormal grains nothing can be said at present.

DISCUSSION

Abnormalities in pollen grain formation.

Multinucleate pollen grains have been observed by De Mol (1923, 1929) and Stow (1930) in *Hyacinthus orientalis* and *Tulipa* sp. Moffett (1932) has observed similar multinucleate giant grains in Kniphofia. He found that the number of abnormal pollen grains decreases higher up the spike. In the majority of cases he found giant pollen grains with four haploid nuclei. This he ascribed to failure of cell-wall formation resulting from the failure of spindle mechanism. He suggested that a genetic developmental reaction determines the failure of the spindles. In support of this view he has referred to the observations of Honing (1923), who has shown that the character 'deformis' in the species *Nicotiana deformis* only shows itself to the full under certain environmental conditions. Thus the 'deformis' abnormality becomes more marked in the late development of the plant. According to De Mol multinucleate condition of the pollen grains is due to excessive development

of normal tetrad cells due to some physiological stimulus inducing further splitting of the chromosomes. Kihara and Lilienfeld (1936) have observed giant multinucleate pollen grains in the F_1 progeny of *Aegilops caudata* × *Ae. speltoides*. They have also explained that the multinucleate condition is due to complete or partial failure of wall formation in the pollen mother cells. They had been able to establish that some of these giant grains were viable and may have tetraploid chromosome number. They mention further that the formation of giant pollen grains is to a great extent dependent on external conditions. They have criticized the view held by Moffett that there is a genetical factor associated with particular environmental conditions leading to failure of spindle formation and consequently the formation of giant grains. Such a factor would be of no ecological value, however, because the plant in question is normally sterile and propagated by vegetative means.

Neither Moffett (1932) nor Kihara and Lilienfeld (1936) have given any satisfactory explanation of the origin of more than four nuclei in such giant grains. It will be seen from the present account that as many as six to eight nuclei have been observed in some of the giant grains. From the evidence given before and judging from the general size relation of the nuclei and the number of nucleoli in these giant grains, it seems obvious that further division of the nuclei must have taken place to account for this increase in number of the nuclei and the nucleoli.

It has been mentioned before that the material for the present study was collected late in the season (beginning of October). We know that various kinds of abnormalities and degenerative changes are commonly met with in the pollen mother cells of Oenothera species when the plants have been exposed to early frost. A number of investigators (for reference see Politzer, 1934; Sax, 1937; Shinke, 1939; and Bhaduri, 1939) have shown that change in the external conditions, especially temperature, may upset the spindle mechanism and consequently the cell-wall formation. Till further observations are made this year, it cannot be said with any surety whether this abnormal behaviour during tetrad formation is due to external conditions alone. Unlike the case of Aegilops hybrids, as observed by Kihara and Lilienfeld (1936), it must be pointed out here that pollen grains produced late in the season are most likely non-viable.

Analysis of size relations of nucleoli.

It has been shown during the present observations that of the four nucleolar chromosomes one pair form a ring bivalent and the other pair is involved in the ring of six chromosomes. It has also been shown that there are three different sizes of nucleoli, one big, one small, and two intermediate, produced by these two pairs of nucleolar chromosomes. In difficult material like Gaura it is not possible to identify from direct observations which of the four different chromosomes is responsible for each of the four nucleoli. Indirect, though conclusive, evidence as to the homology of the nucleoli and the

corresponding chromosomes can, however, be brought forward from a statistical analysis of the particular size combinations of nucleoli in pollen grain nuclei. It has been pointed out before that more than two nucleoli have never been observed in normal haploid nuclei. It is evident, therefore, that the two pairs of nucleolar chromosomes disjoin regularly and the two nucleolar chromosomes in the ring of six take up alternate positions, according to theoretical expectations.

Now, as the four nucleoli of three different sizes form two pairs, there can be only two possible size combinations. Either the large and the small make one pair and the two intermediates the other pair or the large and the small each with one of the two intermediates form the two pairs. According to the first type of combination, 50 per cent. of the pollen grains would show large and intermediate and the other 50 per cent. small and intermediate nucleoli in the pollen grain nuclei. According to the second type of combination, however, large and intermediate, small and intermediate, large and small, and two intermediates are the four possible combinations and each kind will be represented by 25 per cent. of the total number of pollen grains formed. It must be pointed out here that it is very difficult to distinguish accurately, under the microscope, such combinations as large and intermediate from small and intermediate. The presence of two nucleoli of equal sizes, that is the two intermediates, can, however, be identified very easily. Their occurrence at once suggests that the distribution of the nucleoli is according to the second type as described above. Due to lack of material and considerable amount of abnormalities present during the tetrad formation, a proper statistical analysis of the nucleolar sizes according to the above scheme could not be made. The definite observation of a number of pollen grains with two nucleoli of equal sizes (Fig. 25) shows, however, that the large and one of the two intermediates and the small and the other intermediate nucleoli constitute two homologous pairs corresponding to the two homologous pairs of chromosomes with secondary constrictions. The significance and scope of such an analysis of nucleoli are quite obvious. In conjunction with other cytological observations, such an analysis might lead to the identification of a particular chromosome pair or a particular catenation referable to a particular complex of an Oenothera species.

Chromosome catenation and its significance.

Association of chromosomes in rings, instead of bivalents, during diakinesis is now well known in different plant and animal genera. Ring formation in diploids can now be classified into three groups as follows:—

   (*a*) Ring formation in naturally occurring forms: e.g. Oenothera, Campanula, Humulus, Rhoeo, &c.
   (*b*) Ring formation induced after irradiation: e.g. *Zea Mays* (McClintock, 1931), Triticum (Katayama, 1935), Oenothera (Catcheside, 1935), Oryza (Parthasarathy, 1939), &c.

(c) Ring formation after hybridization between homozygous species or races which otherwise show normal bivalents: e.g. Datura, Oenothera, Pisum, &c.

Belling (1925) first suggested interchange of segments between non-homologous chromosomes as an explanation for the ring formation. Belling and Blakeslee (1926) later inferred the occurrence of an interchange of segments between non-homologous chromosomes, to explain the ring of four chromosomes observed by them in trisomic forms of Datura. Darlington (1937) has explained the formation of a ring of fourteen chromosomes in Oenothera on the assumption of six such interchanges, the association of all the interchanged segments, the formation of chiasmata in them, and their subsequent complete terminalization. According to Catcheside (1931) pairing in such ring-forming forms takes place during meiosis between homologous segments of non-homologous chromosomes.

Although this segmental interchange hypothesis can adequately account for ring formation in all the three classes mentioned above, it is still a matter of conjecture as to the exact mode of origin of those naturally occurring forms where chromosome ring formation is a constant feature in diakinesis. In any species one or more segmental interchanges between non-homologous chromosomes, leading to the formation of a ring during diakinesis, will in the first generation produce heterozygotes. These heterozygotes in the next generation will segregate into homozygous new and old forms unless there is a balanced lethal mechanism (Muller, 1918) to prevent homozygous combinations of gametes. In the case of Oenothera, a balanced lethal system combined with large linkage groups associated with ring formation eliminates the survival of the homozygous combinations of gametes and thereby maintains the permanent heterozygous condition. As to the origin of heterozygous forms of Oenothera, however, it cannot be said definitely whether they arose through a number of simultaneous interchanges or whether the homozygous segregates combining with the heterozygotes gradually established the permanent heterozygote through later development of a balanced lethal mechanism. It is difficult to conceive the evolution of the balanced lethal system in the heterozygote simultaneously with the first interchange.

In the family Onagraceae, chromosome catenation has until now only been reported in Oenothera and Gaura. From an unpublished report by Burkert (cf. Gates and Ford, 1938) we know *G. biennis* shows a ring of fourteen and *G. Lindheimeri*, on the other hand, shows seven free bivalents during diakinesis. During the present investigation, however, a definite catenation of $(6)+4_{11}$ has been established in the species *G. Lindheimeri*. It is interesting to point out in this connexion that the catenation of $(6)+4_{11}$ has not been observed in any naturally occurring species of Oenothera, except in one plant in a Californian strain, Hall 34 (cf. Gates and Ford, 1938) which normally shows 7_{11}. In some of the large-flowered Oenothera species like *O. argillicola* or in some strains of *O. franciscana* a catenation of $(4)+5_{11}$ has been recorded

(cf. Gates and Ford, 1938). On the other hand, in some of the different mutations of *O. Lamarckiana* (normal catenation $(12)+1_{11}$) a catenation of $(6)+4_{11}$ has frequently been observed (cf. Gates and Ford, 1938). In a mutation of *O. biennis* (normal catenation $(8)+(6)$), mutation 'Sulfurea', Cleland (1928) has observed a catenation of $(6)+4_{11}$. Catcheside (1935) has also found this catenation of $(6)+4_{11}$ instead of normal 7_{11} in a mutation of *O. blandina* (produced by X'-rays) showing the normal phenotype.

It has been mentioned previously that material for the present study was collected from a single plant. Considering the observations of Burkert, the marked irregularities in the pollen-grain formation, non-setting of seeds, as well as the evidence put forward above, it seems very likely that the plant under investigation might be a mutation. The present doubt will be cleared, however, by an examination of a number of plants grown from seeds.

Significance in Gaura of the relation of chromosomes to nucleoli.

The presence of four nucleoli corresponding to four chromosomes with secondary constrictions in *G. Lindheimeri* recalls the observation made in Oenothera (Bhaduri, 1940). Exactly the same observation has been made both in different heterozygous species of Oenothera like *O. Lamarckiana*, *O. ammophiloides*, *O. Hazalae*, &c., as well as in homozygous species like *O. Hookeri*. It has been shown further that whereas the heterozygous species of Oenothera had four nucleoli of three different sizes, the homozygous species had only two different pairs instead. In *G. Lindheimeri* it has been shown that the size relation of the nucleoli is the same as that of heterozygous Oenotheras and therefore shows the heterozygous condition of this species. There is reason to believe (Bhaduri, 1940) that this particular condition of four nucleoli and their relation to the four chromosomes with secondary constrictions in Oenothera is an older character than chromosome linkage, which has arisen later in the genus. On the present conception of the theory of the rule of correspondence between the number of nucleoli and the number of Sat-chromosomes, four nucleoli corresponding to four chromosomes with secondary constrictions is a condition derived from a previous condition of two nucleoli and two corresponding chromosomes. Whether this step has been brought about in the case of *G. Lindheimeri* by secondary polyploidy or by mutation remains to be settled by further information regarding the relation of chromosomes and nucleoli in general.

On the whole, a marked similarity in the cytological conditions of *G. Lindheimeri* to *Oenothera* species is quite apparent from the present observations and confirms the view of the close affinity of these two genera. In the phylogenetic scheme of Johansen (1929) he has derived both these genera, with a number of other genera as well which he calls 'Oenothera-stem', from a hypothetical ancestral form. Considering all the evidence put forward here, a common ancestry of the two genera appears to be very likely.

SUMMARY

Gaura Lindheimeri has $2n = 14$ chromosomes. The chromosomes can be classified into groups similar to those in Oenothera species. There are four chromosomes with secondary constrictions in the somatic complement which are homologous to the Sat-chromosomes of other plants. The secondary constrictions of chromosomes are well marked, nucleolar and Feulgen positive. Corresponding to these four chromosomes there are, in the somatic nuclei, four nucleoli of three different sizes, one large, one small, and two intermediate, as characteristic also of heterozygous species of Oenothera. From an analysis of the combinations of nucleolar sizes in tetrads and pollen grains a new line of investigation, which will provide corroborative evidence to establish homology of the nucleoli and the nucleolar chromosomes, has been described. The scope of such a study in Oenothera has been pointed out.

The plant under investigation showed a maximum catenation of $(6)+4_{11}$ during diakinesis. The ring of six contains one pair of nucleolar chromosomes and therefore is found always attached to the fused nucleolus. The other pair of nucleolar chromosomes form a free ring-bivalent which is also sometimes found attached to the nucleolus. On various grounds pointed out in the text as well as for the simple reason that this catenation of $(6)+4_{11}$ has not been observed in any of the naturally occurring species of Oenothera, but only in some of the mutations of different species, it has been suggested that this plant might also be a mutation showing the same phenotype as the parent species.

The somatic nuclei show the presence of true chromocentres as also found in Oenothera.

Various abnormalities during pollen-grain formation have been described. The giant pollen grains sometimes show the presence of 1 to 8 nuclei with as many or more nucleoli. This multinucleate condition is due to extra divisions of the nuclei followed by complete failure of cytokinesis. It cannot be said at present whether such abnormalities are due to environmental conditions alone or if genetical factors are involved in this manifestation.

The cytological observations of the present investigation show on the whole the close resemblance between this species and most of the heterozygous species of Oenothera. This lends conclusive evidence to the view, already held by some authors from morphological observations, that the two genera Gaura and Oenothera are phylogenetically closely allied and may have a common ancestry.

In conclusion I desire to express my thanks to Professor R. R. Gates, for his helpful suggestions and encouragement throughout the course of this investigation.

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On the Expression of Sap by Low Pressure[1]

BY

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AND

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With three Figures in the Text

I. INTRODUCTION

IN two recent papers (Phillis and Mason, 1937; Mason and Phillis, 1939) we suggested that the contents of the vacuole can be expressed by rapid application of pressure to carefully packed leaves. The importance of avoiding shearing forces during expression was emphasized. Sap was yielded by the residue only after it had been killed by freezing, &c. This sap, it was thought, was derived mainly from the protoplasm. The vacuolar sap was clear and colourless, while that from the protoplasm was reddish-brown or reddish-yellow. The vacuolar sap had, moreover, a much lower solute concentration and a higher pH value than that presumed to have come from the protoplasm.

In our previous work we did not take account of the possibility that changes might occur in the protoplasm as a result of the application of pressure. It is known that protoplasm is relatively insensitive to pressure exerted uniformly upon it and extremely sensitive to uneven pressures (Heilbrunn, 1937); hence the importance of avoiding shearing during the expression of the vacuolar sap. Moreover, the effects of high uniform pressures are comparable with those obtained by small uneven pressures. Lepeschkin (1937) has pointed out that the first effect of pressure injury would appear to be vacuolar contraction, that is, an enlargement of the protoplasm at the expense of the vacuole, often

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[1] Paper No. 25 from the Physiological Department of the Cotton Research Station, Trinidad.

without any change in cell volume. When the pressure causing vacuolar contraction is prolonged, a second phenomenon may make its appearance. This is the separation of water from the protoplasm to form small vacuoles scattered throughout the protoplasm. Lepeschkin remarks that this process may proceed so far that the protoplasm resembles a foam.

It would seem, therefore, that we must envisage the possibility of at least three different types of sap. The first, *vacuolar sap*, is obtained by rapidly pressing carefully packed leaves at relatively high pressures. The second, *injury sap*, may arise after a variable period of time as a result of pressure, especially low pressures maintained for a long period. It may be·derived partly from the vacuole and partly from the still living protoplasm through changes like those described by Lepeschkin. When the residue, left after the vacuole has been expressed, is killed, a third type of sap is obtained. This may for convenience be termed *death sap*, and, if the vacuole has all been previously expressed, is entirely protoplasmic in origin.

Since the publication of our work Bennet-Clark and Bexon (1939) have compared the effects of slow and rapid pressures on sap expression. They state that they took care to avoid slipping of the leaves. They used Copper Beech leaves which contain anthocyanin in the vacuole but not in the protoplasm of the epidermal and palisade tissues. With rapid (i.e. high) pressure they obtained nearly all the anthocyanin, while on pressing the frozen residue only traces were obtained. They thus confirm our suggestion that with rapid pressing the contents of the vacuole can be obtained. They also extracted sap by slowly pressing carefully packed leaves at low pressure. This sap was colourless. It also had a lower osmotic pressure than the sap obtained by rapid pressing. They concluded that the low-pressure sap was obtained from the vacuole by filtration through the protoplasm. They did not consider the possibility that it might have been produced as a result of injury to the protoplasm. The primary object of the present paper is therefore to consider the nature of the sap produced at low pressures.

II. Experimental

A. *Procedure.*

Leaves of cotton were collected and thoroughly mixed after the large veins had been removed. Three samples of 100 gm. each were then carefully arranged to form wads 4 in. square. They were wrapped in cloth and placed in hydraulic presses. The subsequent treatments of the three wads were as follows:

(1) *Rapid-High-Pressure.* The load was increased to a dial reading of 7,500 lb. in three minutes and by a further 2,500 lb. in each subsequent three-minute interval to 20,000 lb. Separate sap fractions were collected for the three-minute intervals. The weight of sap in the cloth was included in the first fraction.

(2) *Slow-Medium-Pressure.* The load was raised to 6,250 lb. in two minutes. It was kept at this pressure and separate sap samples were taken for each

ten-minute interval for a period of nearly four hours. The sap obtained in the first ten minutes was collected in three sub-fractions of three, three, and four minutes duration respectively, so that some knowledge of behaviour could be obtained during this period of rapid flow. The weight of sap in the cloth was again added to the first fraction.

(3) *Slow-Low-Pressure.* The load was raised to 2,500 lb. in two minutes and kept constant at this pressure. Separate sap samples were again taken at ten-minute intervals and the pressure was maintained for nearly six hours. No sap dripped from the wad for forty minutes, but sap was expressed from the leaves and absorbed by the cloth before any left the wad. It has been found that about 2·5 gm. of sap are required to saturate cloths of the size used in these experiments, but the sap in the cloth has not been included in the first fraction of this treatment since it is uncertain when it was first expressed. Sap was also expressed from frozen material.

B. *Results.*

(1) *Rapid-High-Pressure Treatment.* This treatment was the same as that used on previous occasions for the expression of vacuolar sap. The *total* weights of sap expressed are plotted against the load in Fig. 1. The sap fractions for each 2,500 lb. increase in load do not form a geometric series as in our second paper on sap expression (Mason and Phillis, 1939). The curve calculated from the first three fractions is also shown in Fig. 1. It will be seen that the last three fractions depart from this curve.

In our first paper (Phillis and Mason, 1937) on the extraction of vacuolar sap we found that the concentration of solutes in successive fractions of the high-pressure treatment remained relatively constant. In Fig. 3 it will be seen that the concentrations of chlorine in the first three fractions of this treatment were relatively constant. It will be observed that the concentrations rose sharply in the last three fractions. The changes in pH were essentially similar. It may be significant that the weights of the fractions (see Fig. 1) departed from the calculated curve at the same time that the chlorine concentrations rose. It would seem that the first three fractions represent the true vacuole and that in the later fractions the vacuolar sap became contaminated with sap of higher concentrations separating from the protoplasm. The first three fractions were clear and colourless, while the later fractions, though clear, had a slight pink colour. This colour change is possibly due to the change in pH, for the vacuolar sap reddens on the addition of acid.

The percentage of total water in the cell that is located in the vacuole may, as we have shown (Mason and Phillis, 1939), be calculated on the one hand from the weights of sap water in successive fractions (see Fig. 1), and on the other from the concentrations in the vacuole, the protoplasm, and the whole cell. The vacuolar estimate from the weights of sap comes to 34·4 and from the concentrations to 39·9. The agreement is not as good as that previously found.
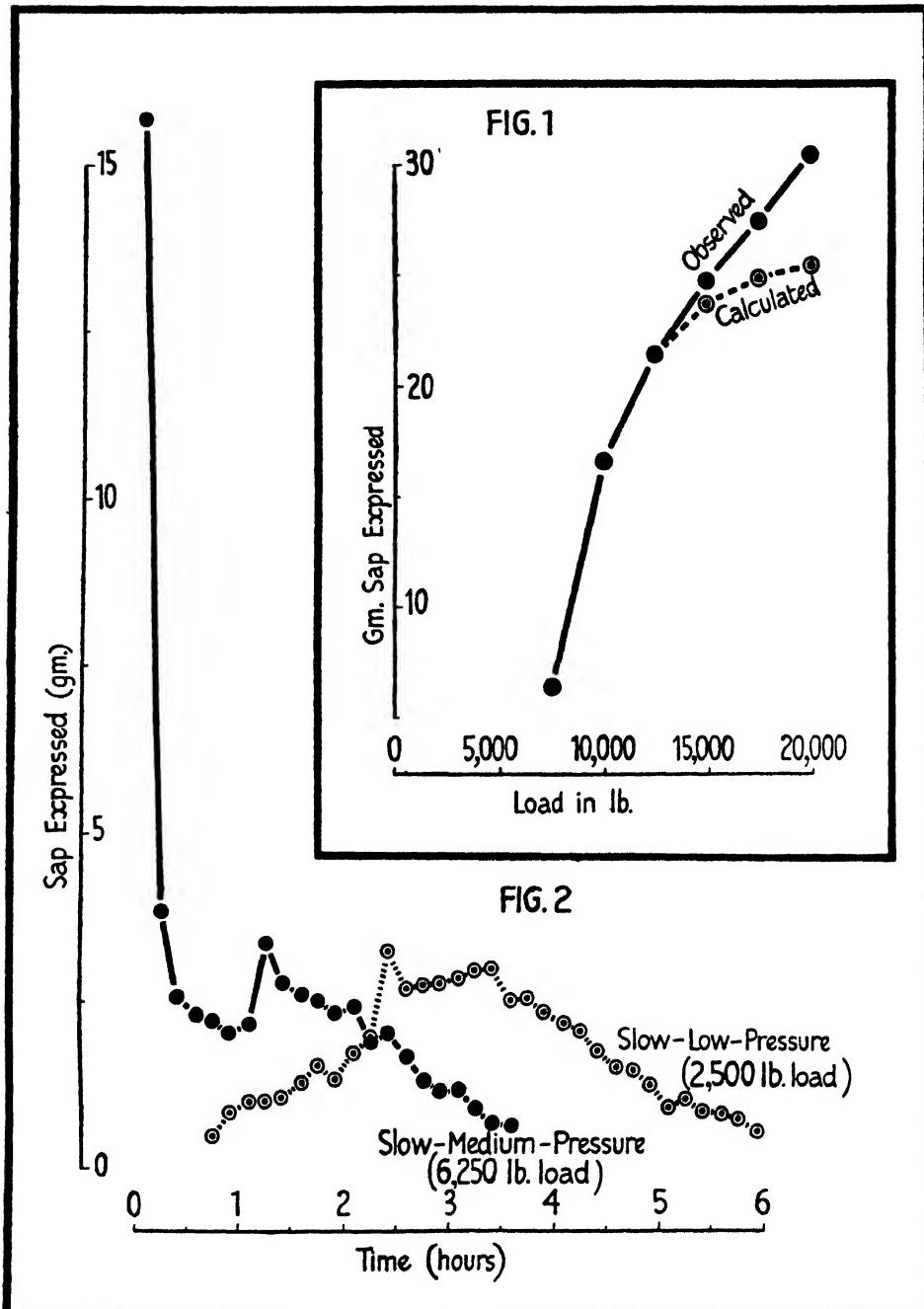
FIGS. 1 and 2. Fig. 1 (inset). Rapid-high-pressure treatment: *total* weights of sap plotted against load in lb., the calculated curve as a broken line and the observed curve as a continuous line: Fig. 2. Slow-medium-pressure and slow-low-pressure treatments: weights of sap expressed during each ten-minute interval plotted against time.

(2) *Slow-Medium-Pressure Treatment.* The weights of sap expressed in this treatment are shown in Fig. 2. They are presented as the weights expressed during each ten-minute interval and not as the *total* weights, as in Fig. 1. The concentrations of chlorine and the pH in each fraction are shown in Fig. 3. They are plotted against the weights of sap. It will be recollected that the first ten-minute fraction was collected in intervals of three, three, and four minutes (cf. Figs. 2 and 3).

Initially, the weights of sap declined rapidly as in the rapid-high-pressure treatment and then rose to a secondary maximum before entering on a slow decline. The concentrations of chlorine and the pH of the first three sub-fractions were approximately constant and then rose steeply. The two curves ran parallel until 26 gm. of sap were expressed; after this the chlorine concentration continued to rise steeply while the pH rose very slowly until 52 gm. of sap had been expressed. The three sub-fractions of the first period were clear and colourless while the sap for the next seven periods was opalescent. Later fractions were clear again but coloured, the colour changing from light yellow to golden brown as pressing proceeded.

To explain these results we suggest that the three sub-fractions collected during the first ten minutes (Fig. 3) represent the true vacuole. Their chlorine concentrations and pH remained unaltered. It will be noted that the chlorine concentrations and the pH values were the same as the first three fractions of the rapid-high-pressure treatment. After this the vacuolar sap became contaminated with sap of higher concentration released from the pressure-injured protoplasm. The appearance of opalescence may be associated with vacuolar contraction. The cause of the divergence in the pH and the concentration curves is not clear.

It is worth noticing that the total weight of sap expressed by this treatment from 100 gm. of fresh material was 56·8 gm., while the weight expressed from the same weight of fresh material killed by freezing was 60·5 gm. In other words, nearly the whole of the sap in the tissue was expressed by prolonged low pressure. It seems clear that much of this sap must have come from the protoplasm and cannot therefore be wholly accounted for as filtration sap from the vacuole. The nature of the chlorine concentration curve is, moreover, unlike that which would be expected from any process of simple filtration of solutes.

(3) *Slow-Low-Pressure Treatment.* It will be seen (Fig. 2) that no sap was obtained until a period of about forty minutes had elapsed. A small amount (about 2·5 gm.) of sap was yielded by the leaves and absorbed by the cloth before sap flowed from the wad. The rate at which sap was released increased at first, but later, after a relatively steady period, began to decline. The total weight of sap yielded was 58·5 gm., which was nearly as great as that obtained from the leaves killed by freezing (60·5 gm.). The sequence of events indicates that some change was taking place in the protoplasm. There was apparently a slow separation of water and at the same time probably an increase in the ease

with which water could move through the protoplasm. Throughout the whole period sap was yielded at much slower rates than in the case of material killed by freezing. The protoplasm, in short, offered a much greater resistance to the movement of water than is the case in dead material.

The chlorine concentrations (Fig. 3) rose rapidly till about the period of maximum sap production. After this period the rate at which they rose was markedly reduced, as was the rate of sap production. The pH values follow closely the changes in chlorine concentrations, changing rapidly at first, then more slowly, but, unlike the chlorine concentrations, there was a further period of rapid change shortly before pressing was discontinued. It will be noticed that the chlorine concentrations at the commencement of sap expression are greater than those of the other two treatments. It is impossible to reconcile this with the *filtration* hypothesis. Bennet-Clark and Bexon, on the other hand, found that their low-pressure sap had a lower concentration than their high-pressure sap. It is possible that their vacuolar sap may have been contaminated with *death* sap due to shearing. The first six fractions in our experiment were brown and not very clear. After this there was a sudden change from brown to clear gold. It should be pointed out that the continued increase in chlorine concentration is quite unlike the concentration changes in successive sap fractions expressed from material killed by freezing; in material that has been killed the concentrations remain relatively constant.

III. Discussion

There would appear to be two possible explanations of the origin of sap produced by low pressures, viz. injury and filtration. Bennet-Clark and Bexon believe that it is due to *filtration* of the contents of the vacuole through the protoplasm. It is not altogether clear how, as a result of filtration, *chlorides* could be retained and *liquid water* allowed to pass. It is also difficult to see how hydrostatic pressure could be generated and maintained inside a membrane which allowed liquid water to pass through as a result of pressure. For liquid water to traverse a membrane, and in the case of a vacuolated plant cell the whole protoplasm may be regarded as a membrane, either a continuous aqueous phase or a porous structure would be required. We have already pointed out that our results favour the view of Lepeschkin that there is no continuous aqueous phase in protoplasm. Water appears to be present in protoplasm as water of constitution.[1] That *liquid protoplasm* is porous seems improbable. Water moves through protoplasm, we think, as molecular and not as associated water. The movement of hydrate water in a crystal or the

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[1] A union which appears to require a continuous supply of metabolic energy for its maintenance. An example of this type of reaction is to be found in the formation of dianthracene from anthracene, and its maintenance in this form under the influence of light. When the light is removed the polymer reverts to its original components.
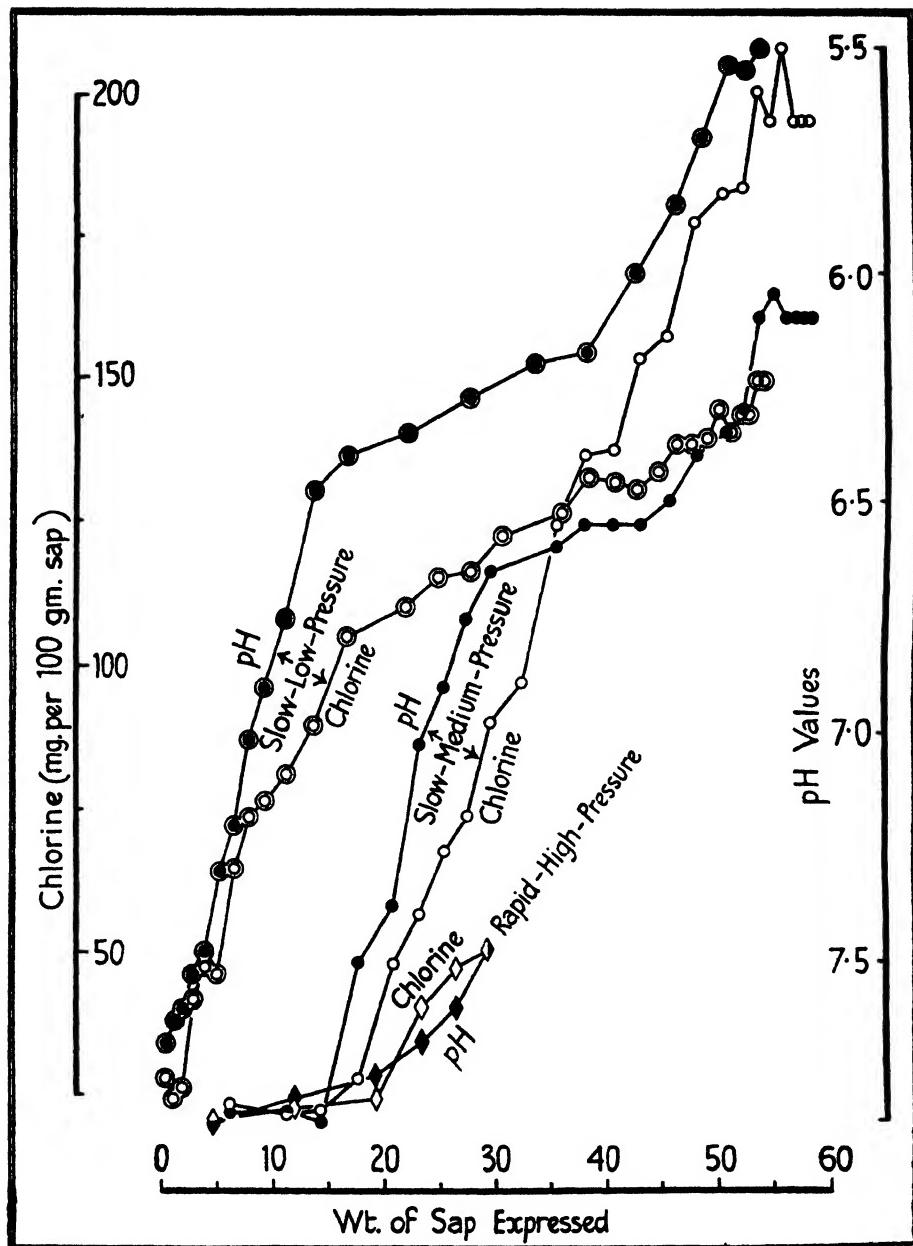
FIG. 3. All treatments: chlorine concentrations and pH values (scale inverted) plotted against weights of sap expressed.

diffusion of water through a liquid with which it is immiscible are instances of such movement.

Bennet-Clark and Bexon were led to postulate 'filtration' for two reasons. Their leaves contained anthocyanin in the vacuole and not in the protoplasm. With rapid-high-pressure extraction they obtained nearly all the anthocyanin. From this they inferred, correctly we believe, that they had extracted the vacuole. With slow-low-pressure they obtained a colourless sap. Their conclusion that it came exclusively from the vacuole as a result of filtration of the anthocyanin we believe to be incorrect. The more probable explanation is, we think, that the anthocyanin was water-soluble but was not soluble in the protoplasm, so that it was not taken up by the protoplasm when vacuolar contraction and dispersion of the vacuole in the protoplasm took place. When the subsequent stage of pressure injury—vacuolation—set in, the new vacuolar fluid would not contain anthocyanin. Their other reason for believing that filtration took place was that the osmotic pressure of the slow-low-pressure sap was less than that found in the high-pressure sap. We have already suggested that they may have had some shearing in the wad and consequent contamination of their high-pressure sap by *death* sap.

A number of reasons for believing that the low-pressure sap arises as a result of injury to the protoplasm and not as a result of filtration have already been adduced. A rather striking experiment which points in the same direction may, however, be mentioned. Very young cotton leaves at a stage before the lamina had flattened were carefully packed and placed in two hydraulic presses. In one the pressure was rapidly increased to 20,000 lb. This treatment is that normally used by us for the expression of vacuolar sap. No sap was obtained even when this pressure had been attained. As the leaf material used may well not have begun vacuolation, this result was not unexpected. In the other wad a constant pressure of 2,500 lb. was maintained and sap began to flow from the wad after a period of one hour. This experiment thus tends to confirm the view that prolonged pressure causes injury to the protoplasm and leads to the separation of water. Incidentally it also indicates that the sap expressed by the rapid application of high pressure is derived from the vacuole.

Susceptibility to pressure injury seems to be very variable. Thus Lepeschkin (1927) reports that in some Spirogyra threads a single blow caused coagulation in over 50 per cent. of the cells while other threads required 350 blows to cause 25 per cent. of the cells to coagulate. In the case of cotton leaves of the same age, the stability of the protoplasm to pressure injury has been found to be very variable. Thus at certain times it has been found impossible, using the rapid-high-pressure treatment, to obtain sap of constant solute concentration. This was notably so after the onset of the rains at the close of a rather severe dry season. At other times, sap of constant solute concentration is readily obtained. Again with some plants sap of constant solute concentration is readily obtained, while with other plants even the

second fraction may show a well marked increase in concentration. These apparent variations in the stability of protoplasm to pressure injury are at present the subject of further inquiry.

IV. SUMMARY

1. When leaves of cotton are compressed under pressures too low to cause immediate expression of the vacuolar sap, a sap is expressed, after varying time-intervals, which differs from the true vacuolar sap.

2. When such low pressures are maintained, almost as much sap can be expressed from the leaves as can be expressed from previously frozen material, but at all times the rate of flow from such *living* material is far less than from killed leaves.

3. Neither the volume of sap which can be obtained under this low-pressure treatment nor the concentrations of chlorine in successive fractions is in harmony with the view that this sap is produced by the filtration of the *vacuole* through the retaining layer of protoplasm.

4. It is considered that this low-pressure sap is derived from *injured protoplasm*, possibly by some such process as vacuolation.

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A Method for the Continuous Measurement of Transpiration of Single Leaves under Natural Conditions

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With nine Figures in the Text

INTRODUCTION

AS Maximov (1929) has pointed out, there are three possible methods whereby the rate of transpiration can be measured, the determination of the water vapour transpired being theoretically the most satisfactory; but although many attempts have been made to use this method none has been fully successful.

Any method for measuring transpiration must fulfil the first three conditions following, and should, if possible, fulfil the fourth. (1) The part of the plant being studied should be attached to an intact, rooted plant. (2) The method adopted should not alter the environmental conditions. (3) The transpiration rate should be calculable in absolute units, i.e. weight of water/unit area/unit time. (4) The transpiration rate should be measurable over short intervals of time, preferably continuously.

This paper presents a method which fulfils the above conditions.

THEORY

If a stream of air of known velocity is passed through a small transparent cup attached to the transpiring surface of a leaf, and if the amount of water vapour per unit volume in the air-stream before and after its passage is known, the rate of transpiration can be calculated. If at the same time the velocity of the air be such that the average humidity in the cell be not appreciably raised, then the transpiration rate will differ but slightly, if at all, from that to be expected from a freely exposed leaf. If, at the same time, the temperature and the humidity of the air-stream are uncontrolled (i.e. are the same as ordinary atmospheric air), then the first three conditions above are fulfilled. The method of measurement to be described complies with the fourth condition.

THE METHOD OF MEASUREMENT

If the temperature, pressure, and the saturation deficit of a sample of air are known, the amount of water therein contained is also known. Consequently the difference in the water content of two equal air samples each at the same

SEALING
WAX

Copper

Constantin

MACERATED FILTER PAPER

DETAIL OF WET
THERMOCOUPLE

FIG. I A

AIR

Filter paper

Rubber
Cap

Calibration
marks

FIG. I B

L

L₁

A

B

FIG. I

F

F₁

LEAF CUP
FIG. I C

To suction
pump
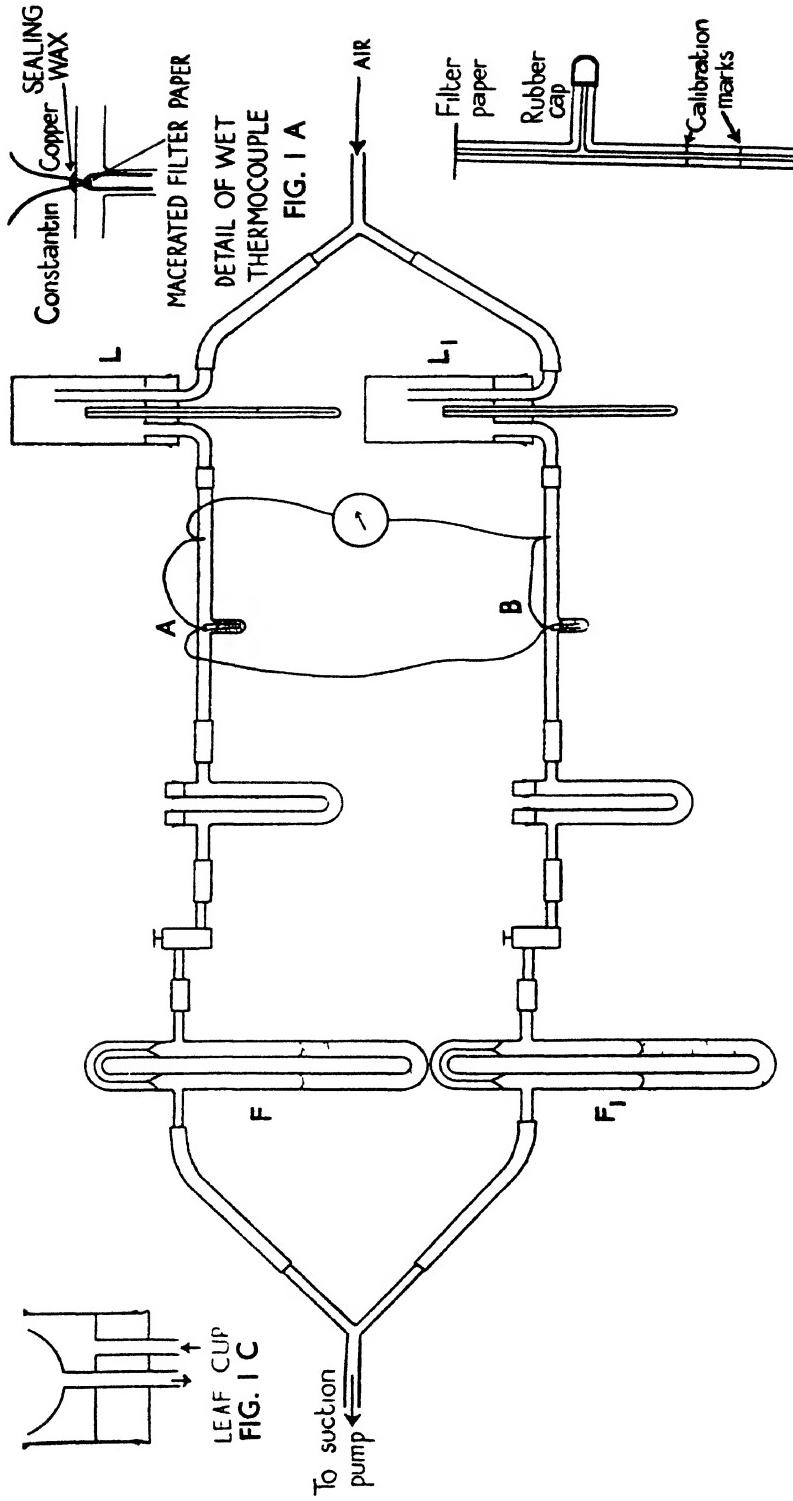
FIGS. 1–1C. Diagram of the apparatus as set up for calibration   1A. Detail of wet thermocouple.   1B. See text.   1C. Leaf cup.

temperature and pressure is proportional to the difference in their saturation deficiencies.

Two streams of air from a common source, controlled by two calibrated flowmeters, are passed through two exactly similar narrow glass tubes, A and B as in Fig. 1, the internal diameter of each tube being 2 mm. and its length 8 cm. A short sidepiece, about 1·5 cm. long, in the middle of the tube is sealed by a rubber cap. Two narrow holes are bored in the tube, one directly opposite the sidepiece, the other about 2 cm. away on the same side. Two constantin-copper thermocouples, one bare and the other covered by a wick, are passed through the holes, centred in the tube, and sealed in place by a drop of sealing-wax. The wet thermocouple (the wick of which hangs in the side-piece) is prepared by tying an oil-free cotton thread round the thermocouple so that a strand hangs down on either side, as in Fig. 1A, dipping into a water reservoir in the sidepiece. The thermocouple is thinly coated with, and the space between the two threads and the thermocouple filled by, macerated filter-paper boiled with a trace of soluble starch. The four thermocouples are connected as shown in Fig. 1, so that the E.M.F. produced by one pair is counterbalanced by the equal and opposite E.M.F. produced by the second pair. Provided that the rates of air-flow remain equal and constant, this condition of zero E.M.F. is independent of external fluctuations in temperature and humidity over a wide range.

In practice, since the diameter of the two tubes at the point where the air-stream passes the wet thermocouple cannot be measured, and the efficiency of the wicks is also variable, one air-stream is adjusted to a known rate of flow and the second is then altered until zero E.M.F. is produced. An error is thus introduced, for with any change in humidity one thermocouple is affected before the other. This error can be reduced to insignificance by reducing the volume of the tubes leading to the source of supply, and by so making the thermocouples that the necessary difference in the rates of air-flow is small.

A d'Arsonval-type galvanometer, of 34·4 ohms resistance, and sensitivity of 500 mm. deflection for 1 microamp at a working distance of 2 metres from the mirror, has proved very suitable. Water vapour introduced into one air-stream is quantitatively detected by galvanometer deflection.

CALIBRATION

The apparatus was calibrated by introducing known amounts of water vapour into one air-stream. It was set up as in Fig. 1. The glass chambers (L and L_1) are sealed by three-hole rubber stoppers. In each, inlet and outlet tubes pass through two holes, and a sealed capillary tube through the third. One flowmeter (F) was set to a known rate of air-flow, and the air-stream through the other (F_1) adjusted until the E.M.F.'s produced by the two circuits are equal and opposite. When the two circuits have remained in balance for at least ten minutes the capillary tube in the chamber

(L) is replaced by a special capillary tube, illustrated in Fig. 1B, having a side-piece closed by a rubber cap and calibrated between two marks about 2 cm. apart at one end. This is filled with water, passed through the stopper, and capped by a small piece of filter-paper. As air is drawn through the chamber
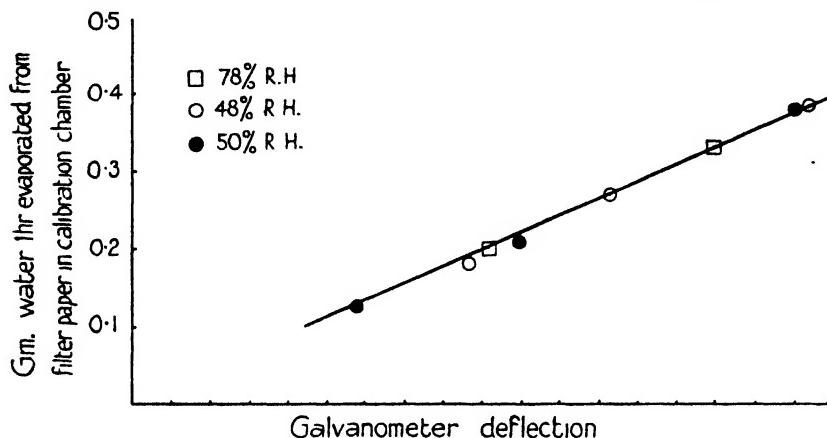
FIG. 2. The relationship between galvanometer deflection and the rate of evaporation of water during calibration at different humidities.

the water evaporated from the filter-paper is replaced from the capillary tube, and the resulting movement of the water meniscus measured by a horizontal microscope.

The air circuits, one of which now contains an additional supply of water vapour, are allowed to settle down once more until a steady rate of travel of the water meniscus and a steady galvanometer deflection are attained.[1] Galvanometer readings are now taken at 30-sec. intervals. The average distance travelled by the meniscus during the same periods is also calculated. This is repeated several times, the meniscus being returned to its original position by a screw clip on the rubber cap of the sidepiece. This procedure is repeated for different sizes of filter-paper and at different humidities, the rate of flow in each unit being constant at constant temperature.

In the Plant Physiology laboratory of this Station the air temperature varies only about 2° C. throughout the day, and calibration curves under such conditions and between such widely differing humidities as 50 per cent. R.H. and 80 per cent. R.H. are coincident straight lines. Hence, as would be expected from theoretical considerations, the apparatus is independent of changes in atmospheric humidity at least between 50 per cent. R.H. and 80 per cent. R.H.

Typical calibration data are given in the table on p. 29 and illustrated in Fig. 2.

[1] If ordinary atmospheric air is used both the rate of travel of the meniscus and the galvanometer deflection will be variable owing to normal fluctuations in atmospheric humidity. The results, however, when plotted on the graph lie on a straight line.

Calibration Data

Diameter of capillary tube .   .   .   . = 0·93 mm.
Amount of water evaporated from filter-paper for 2 mm. move-
   ment of the water meniscus   .   .   .   .   . = $1·359 \times 10^{-3}$gm.
Air-flow through fixed flowmeter   .   .   .   .   . = 9·0 litre/hr.
   ,,   ,,   variable   ,, ... = 8·7   ,,
Humidity .   .   .   . = 50 per cent. R.H.

Time taken for meniscus to travel 2 mm.		Amount of water evaporated (gm./hr.)	Galvanometer deflection (to nearest 0·1 mm.)
min.	sec.		
2	10½	0·03764	17·0
2	10		17·1
2	10		17·1
2	10		17·1
3	54		10·0
3	54		10·0
3	53	0·02091	10·0
3	53½		10·0
3	54		10·0
3	54		10·0
6	27		5·8
6	28		5·8
6	27	0·01264	5·9
6	27½		5·8
6	27		5·8

After this calibration, the glass chambers L and L_1 are replaced by leaf cups as in Fig. 1C, having approximately the same air resistance as the glass chambers. They are first sealed and the apparatus allowed to settle down to its state of zero E.M.F. This may necessitate a very slight adjustment in the flowmeter F_1 since slight changes in the air resistances of the circuits may have been introduced; such adjustment does not materially affect the accuracy of the results. The seal of one leaf cup is removed and the cup attached to the lower side of the leaf by low melting-point wax. Galvanometer readings are now taken every thirty seconds and by reference to the calibration curve the rate of transpiration at any given moment can be determined. Since the area covered by the leaf cup is known, the amount of water transpired can be calculated as gm./unit area/unit time.

By enlarging or reducing the area of the leaf covered by the leaf cup or by varying the rate of air-flow in the units so that the maximum increase in humidity of the air inside the leaf cup is not excessive, the apparatus can be used on plants having high or low rates of transpiration.

Results Obtained by this Apparatus in a Study of Wilting

The changes in transpiration rate accompanying the wilting of a maize leaf are given as an illustration of its utility. Fig. 3 illustrates graphically the changes observed.

The experiment was conducted in a dull diffused light. For a preliminary period of forty minutes the transpiration rate of an attached leaf was measured at thirty-second intervals. This established the general level of the transpiration rate under the prevailing conditions. The leaf was then severed from the
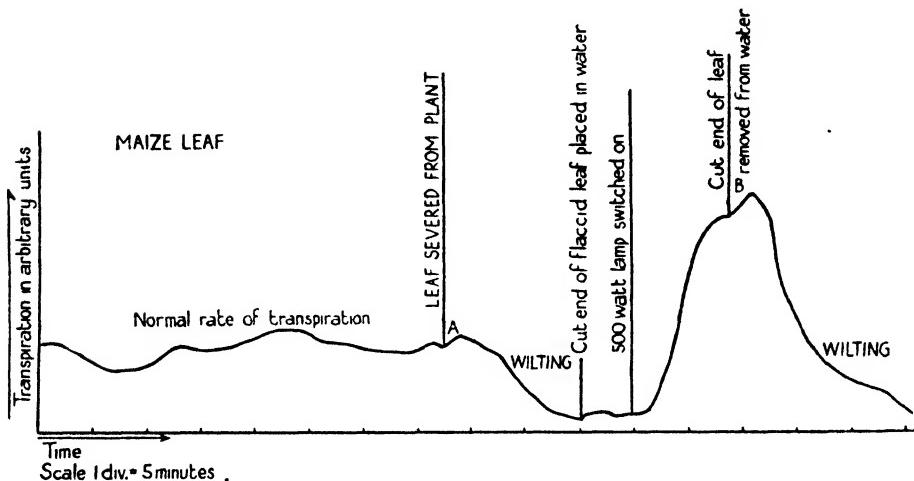
FIG. 3. The normal march of transpiration of a maize leaf under dull conditions, and the transpiration rate during wilting showing the 'preliminary acceleration period' at A and B.

plant. The transpiration rate rose for 2–2½ minutes, and then declined, at an increasing rate, until, when the leaf was quite flaccid, it had reached a very low value. The cut end was placed in water and the leaf regained turgidity. No marked increase in the transpiration rate accompanied this, however; but since recovery took only five minutes this is, perhaps, to be expected. The turgid leaf was then illuminated by a 500-watt lamp placed 2 ft. away, screened by a deep layer of water. The transpiration rate rose slowly at first, and later rapidly. After ten minutes the water-supply to the leaf was interrupted. The transpiration rate rose for three minutes and then declined during subsequent wilting. The temperature throughout the experiment was constantly at $25° \pm 0·5°$ C. and the relative humidity between 70 and 75 per cent.

The phenomena here reported agree with studies of wilting reported elsewhere. The initial rise of the transpiration rate at the beginning of wilting, well shown in these data, is presumably that described by Knight (1922), although it occupies very much less time than he reports for *Eupatorium adenophorum*. The short time-interval before attainment of the maximum transpiration rate after commencement of wilting in *Zea Mays* may well be due to the high temperature at which the experiment was conducted, since Knight has shown 'a distinct relationship between the temperature during the experiment and the length of the interval between the beginning of wilting and the occurrence of the maximum transpiration rate'.

THE APPARATUS IN THE FIELD

The apparatus was tested on *Coffea arabica* in the field. It was enclosed in an insulated, double-walled, wooden box inside a tent with a double roof. Nevertheless, the temperature of the air inside the box varied 10° C. throughout the day, the external temperature at the same time varying 18° C.
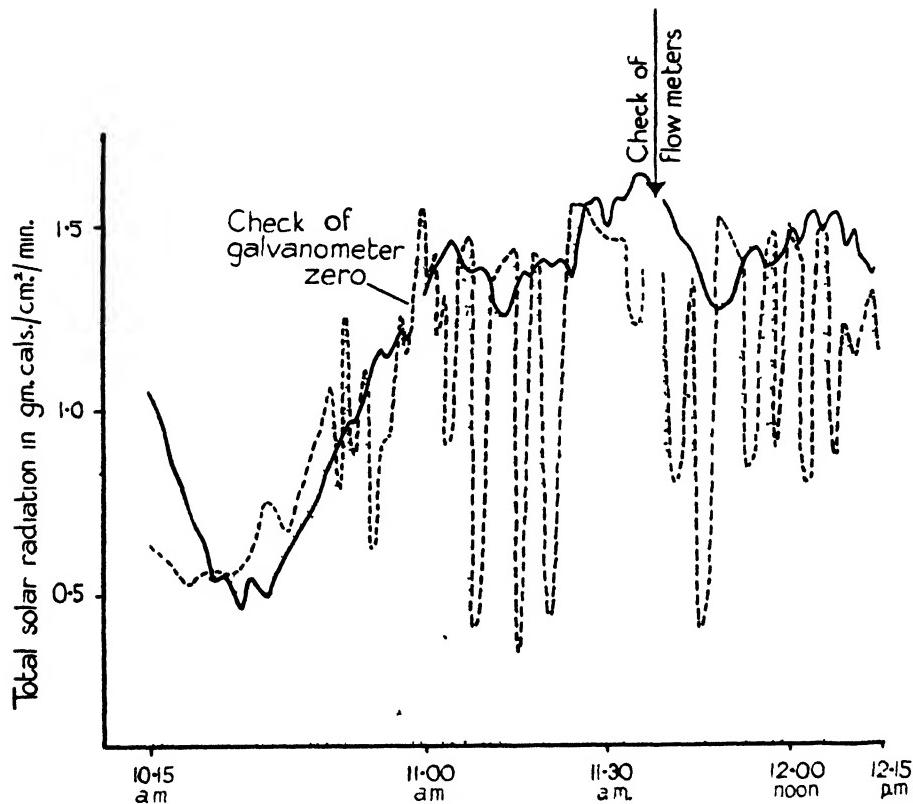
FIG. 4 The transpiration of a portion of a leaf of *C arabica* in the open (continuous line) The vertical component of total solar radiation during the same period is shown by the dotted line

This lack of adequate insulation caused many errors in the working of the apparatus. The galvanometer zero and the flowmeters respond to temperature changes, the maximum error due to this sometimes reaching 20 per cent. By working for periods of 1–2 hours only, during which the external temperature varied only 5° C., and by frequently checking the galvanometer zero and flowmeters, it was possible to obtain results with a maximum error of only 5 per cent. Since under these conditions frequent recalibration is necessary, the facilities for which were lacking, the march of transpiration is shown in the following graphs in arbitrary units. A small portable reflecting galvanometer of 8 ohms resistance and giving 2·1 cm. deflection for 1 microamp was used.

Figs. 4, 6, and 8 illustrate results obtained from leaves of *C. arabica*, the area of the leaf cup in contact with the leaf being 3 cm.[2] Readings of the galvanometer and solarimeter were taken every alternate thirty seconds. The results gave positive values of between 0·56 and 0·59 for the coefficient of
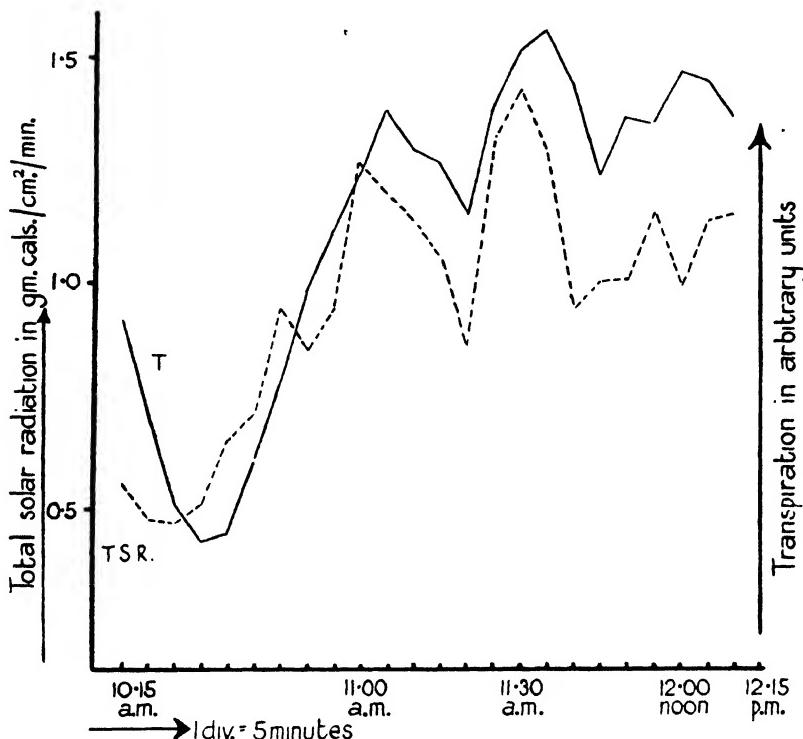
FIG. 5. Averages of the values for transpiration and total solar radiation over successive five-minute intervals.

correlation between solar radiation and transpiration, which are significant to the 1 per cent. point.

Figs. 5, 7, and 9, prepared from the averages of the values of the transpiration and solar radiation over successive five-minute intervals, show more clearly the general outlines of the march of transpiration throughout the experimental periods.

The short experience of this apparatus in the field has not been wholly satisfactory, for reasons already explained. It does enable, however, the detailed march of the transpiration rate of single attached leaves to be studied under uncontrolled conditions, albeit over periods of a few hours only; no other method with which I am acquainted will do even this.

Improvements in temperature control would undoubtedly make the apparatus more suitable for field-work. The outbreak of war has prevented the
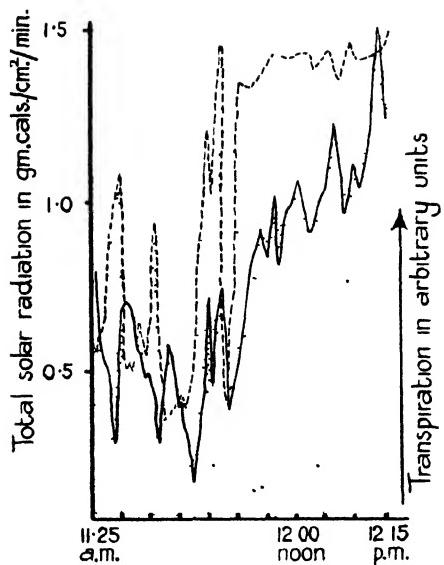
FIG. 6.
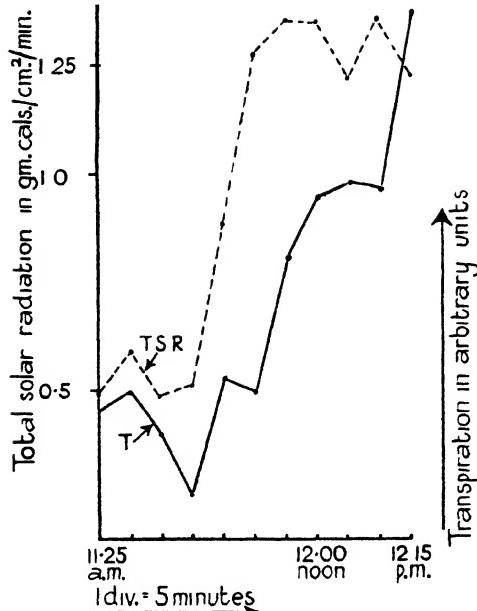
FIG. 7.
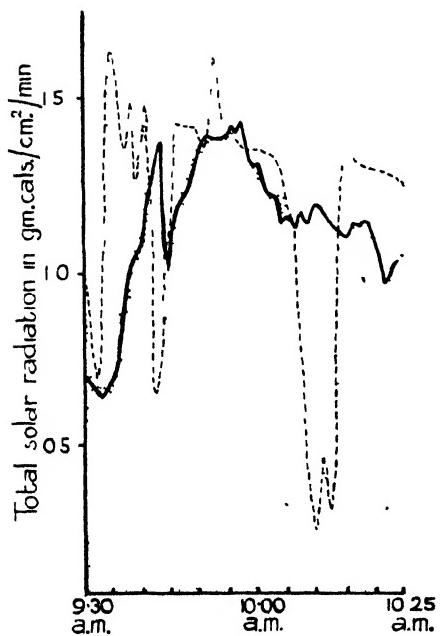
FIG. 8.
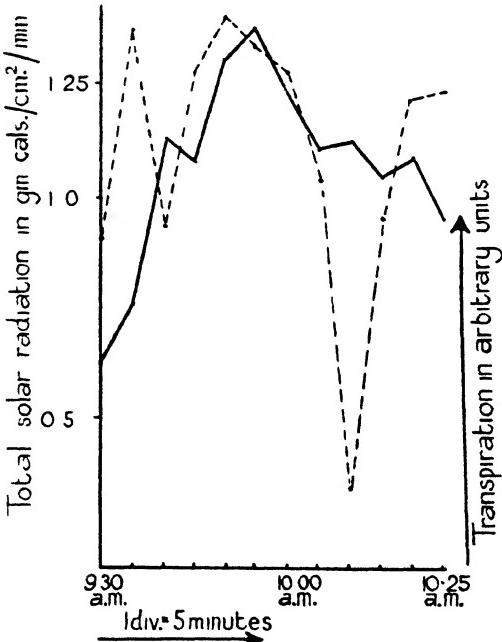
FIG. 9.

FIG. 6. The march of transpiration of a single horizontal coffee leaf in the open, and total solar radiation (dotted lines) over the same period.

FIG. 7. Average of values for transpiration and total solar radiation over successive five-minute intervals.

FIG. 8. The march of transpiration of a single horizontal leaf of *C. arabica* in the open.

FIG. 9. Averages of values for transpiration and total solar radiation over successive

continuation of this work, and the results already achieved are therefore presented somewhat prematurely.

SUMMARY

An apparatus for the continuous measurement of the water vapour transpired by a portion of a leaf under approximately normal environmental conditions is described.

Illustrations of its use in the study of the march of transpiration in *Coffea arabica* and in the study of wilt in *Zea Mays* are given.

ACKNOWLEDGEMENTS

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Further Studies on the Action of Heteroauxin on Phaseolus vulgaris

BY

L. PORTHEIM

With Plate I

THE investigations described in this paper are a continuation of work previously carried out at the Biological Research Institute of the Academy of Science in Vienna (Portheim, 1936 and 1937). The studies in Vienna were chiefly concerned with the action of 0·1 per cent. β-indolyl-acetic acid in lanolin, when applied to the 'Non Plus Ultra' variety of *Phaseolus vulgaris*.

It was shown that *Phaseolus vulgaris* exhibits very definite reactions to treatment with heteroauxin, of the same types as have already been described in the literature as occurring in other kinds of plants after similar treatments. The changes in *Phaseolus vulgaris* induced by the action of heteroauxin are chiefly to be seen in the formation of swellings and curvatures of the petioles and the pulvini, which are normally present at the distal and basal ends of the petiole respectively. The degree and direction of the curvature exhibited by these organs is controlled by the quantity of heteroauxin paste and the exact point at which it is applied. The downward curvature of the lamina, which takes place when the upper side of the joint at the distal end of the petiole is treated with heteroauxin paste, can also be induced when fragments of the paste are applied to the lowest veins of the leaf lobes. The conclusion was drawn that in the latter case the heteroauxin is translocated to the distal joint of the petiole where it operates in the same way as if it had been applied directly in that position. The heteroauxin is thought to control the positions which the leaves assume and also to induce changes in the distal and basal joints of the petiole which are partly responsible for the actual movements.

Further experiments were subsequently made at the Jodrell Laboratory at the Royal Botanic Gardens, Kew, but in most of these the heteroauxin was applied in agar discs at a concentration of about 0·1 per cent. Dwarf French Beans (Carter's 'Canadian Wonder') were used, and were found to react in very much the same way as the variety of beans employed at Vienna.

The previous investigations led to the conclusion that the phenomena induced by growth substances when applied at relatively high concentrations are not only due to growth processes, but also to changes of water-content induced in the organs. In order to investigate this possibility more fully,

petioles of the first leaves of *Phaseolus vulgaris* were used as they were already known to be sensitive to heteroauxin. The lamina was removed from the petiole above the distal joint, thereby preventing translocation. The small amount of assimilates produced by photosynthesis within the petioles and stems, as well as the reserve-substances already present, could have reached the places of consumption in the petioles only in solution. In this way it should also be possible to determine whether the correlative relations of these functionless organs, the decapitated petioles, could be changed by the action of growth substances. This question, concerning the correlation between the petiole without lamina and the abscission layer, had also been studied by Mai (1934) and La Rue (1936).

In the present investigations attempts were also made to ascertain to what extent alterations in water distribution played a part in these phenomena. Mai (1934) states that when the bladeless petioles of Coleus were treated with orchid pollinia a drop of fluid sometimes overflowed the surface of the wound in contrast with the controls in which there were no pollinia. He implies that the sap supply in the treated petioles is increased by the pollinia. This phenomenon was also sometimes to be observed in my investigations with bladeless petioles of the first leaves of *Phaseolus vulgaris*, but so infrequently and indistinctly that no conclusion was possible.

Schlenker (1937) says that it seems possible that in the experiments to induce epinasty by outside stimuli the changes in turgor play a role.

EXPERIMENTS WITH HETEROAUXIN IN LANOLIN

After removal of the lamina, the remaining part of the upper joint and the surface of the wound were painted with o·1 per cent. lanolin-heteroauxin paste, or with lanolin alone. Plants from which the lamina had been removed without other treatment served as additional controls. The control plants of both types exhibited no morphological changes, but swellings, more or less strongly developed, appeared on the petioles treated with the heteroauxin paste either at or below the point of application. In the experiments with 'Non Plus Ultra' beans it had been found that, after treatment, petioles originally in a diagonal position exhibited an upward curvature. In 'Canadian Wonder' beans, on the other hand, similar upward curvatures were less common, but downward curvatures were more frequent. The maximum swelling occurred where the paste was applied to the apical joint in the region of the paste, while it was progressively less pronounced towards the base of the petiole (Pl. I, Fig. 1). In the control plants the distal joints fell off some time after removal of the lamina, but this did not occur after treatment with heteroauxin. The basal joints and adjacent parts of the petioles also became swollen after treatment with lanolin-heteroauxin, but the apical portions of the petioles showed signs of drying up (Pl. I, Fig. 2). In similar experiments in which the distal joints had been treated in the same way, but the plants had been cultivated in a very damp atmosphere under a bell jar,

the petioles became swollen more or less equally throughout their length (Pl. I, Fig. 3).

EXPERIMENTS WITH HETEROAUXIN IN AGAR DISCS

The investigations were repeated using agar discs containing heteroauxin. β-indolyl acetic acid in the proportion of 1 : 1,000 was added to an agar solution and distributed as evenly as possible by stirring with a glass rod and shaking the dish, but complete solution of the heteroauxin was not obtained in spite of these precautions. For this reason the changes induced in the plants may have been due to relatively high concentrations. Discs of agar, 1·2 cm. in diameter, with and without heteroauxin, have been attached to different parts of petioles from which the lamina has been removed. The opposite petiole at the same node was generally left untreated for comparison. The agar discs soon dried up, often after a single night, so that it was impossible to remove them without injuring the plants. A striking change induced by these treatments was that the plant tissues in the region of the agar discs containing heteroauxin sooner or later became somewhat shrunken, especially above the discs themselves. Shrinkage of the petiole was less pronounced in control plants to which discs of pure agar were attached and in untreated petioles, thus demonstrating that stronger contraction of the tissues was caused by the heteroauxin itself. Although this effect came at first as a disagreeable surprise, it subsequently made possible the recognition of the influence of the heteroauxin in attracting water to the petioles.

Agar discs containing heteroauxin have also been attached to petioles after removal of the lamina in the positions indicated in the following table. For the sake of brevity the letters used in the table to denote each type of treatment are repeated in the text below.

A. Agar-heteroauxin discs on the distal joint.
B. ,,   ,,   ,, below   ,,   ,,
C. ,,   ,,   ,, in the middle of the petiole.
D. ,,   ,,   ,, on the basal joint.
E. ,,   ,,   ,, below the distal joint after removal of the latter.

Although experiments have been carried out with many hundreds of bean plants, those which are here described in detail were from investigations made at Kew between February and August 1939, using about 400 plants.

Curvature of the petioles.

With treatment A some upwardly directed curvatures were induced similar to those which previously obtained by applying the heteroauxin in lanolin (Pl. I, Fig. 6). Upward curvatures were never induced in the variety 'Canadian Wonder' by treatments B, C, D, and E, but there were almost

always downward curvatures either above or below the position, of the agar-heteroauxin disc (Pl. I, Figs. 9, 10, 11, 13). The control petioles in most instances became only slightly bent or exhibited no curvature, but in rare instances the bending was more pronounced.

Growth in length of the petioles.

The first experiments dealing with the growth in length of the petioles after removal of the lamina were carried out by attaching to them agar discs containing heteroauxin irrespective of their initial length; later on, however, only the shorter petioles were treated. In this way it was found that petioles treated with heteroauxin increased in length more rapidly than the controls. The number showing a length greater than the untreated 6–9 days after the experiments were as follows:

Agar-heteroauxin on the distal joint, 93·9 per cent.
    ,,   ,,   below   ,,   ,, 100   ,,   ,,
    ,,   ,,   below the distal joint after removal of the latter 100 per cent.
    ,,   ,,   on the basal joint, 85·7 per cent.
    ,,   ,,   in the middle of the petioles, 68·2 per cent.

Measurements of the length of the petioles during the first few days gave the following average-rates of growth, recorded as percentages of the length at the time of the previous measurement:

Experiment 44A. Agar-heteroauxin on the distal joint:
1 day with agar-heteroauxin 18·9 per cent., control 6·7 per cent.
2 days ,, ,,   ,,   3·0 ,,   ,,   ,,   1·7 ,, ,,
5 ,,   ,,   ,,   ,,   1·8 ,,   ,,   ,,   0·5 ,, ,,

Experiment 44 C. Agar-heteroauxin in the middle of the petiole:
1 day with agar-heteroauxin 18·3 per cent., control 5·1 per cent.
2 days ,, ,,   ,,   6·4 ,,   ,,   ,,   1·6 ,, ,,

Experiment 46 E. Agar-heteroauxin below the upper joint after removal of the latter:
1 day with agar-heteroauxin 12 per cent., control 4·2 per cent.

Swelling of the petioles.

In all the investigations with high concentrations of agar-heteroauxin the formation of swellings was induced up to 100 per cent., but in one experiment, with a concentration of 0·025 per cent., swellings were formed on only a maximum of 71·4 per cent. of the petioles (Pl. I, Figs. 8–13). The largest swellings usually appeared on the portions of the petioles below the discs. The following table shows the percentage of petioles which became completely swollen after treatment with heteroauxin. The letters refer to the position in which the heteroauxin was applied as shown in the scheme above.

A. 75   per cent. of petioles treated.
    75 ,, ,, ,, ,,   which exhibited any swelling.
B. 82 ,, ,, ,, ,,   petioles treated.
    92 ,, ,, ,, ,,   which exhibited any swelling.
C. 57·9 ,, ,, ,, ,,   treated.
    64·7 ,, ,, ,, ,,   which exhibited any swelling.
D. 14·3 ,, ,, ,, ,,   treated.
    15 ,, ,, ,, ,,   which exhibited any swelling.
E. 100 ,, ,, ,, ,,   treated.
    100 ,, ,, ,, ,,   which exhibited any swelling.

The petiole swellings increased in size during the first few days, but then some of them shrunk, while the remainder became more completely swollen. Later on all of the swellings contracted owing to shrinkage of the petioles (Pl. I, Figs. 8 and 9). This shrinkage generally became more apparent in petioles which had been treated with heteroauxin than in the controls.

Local shrinkage or drying up could often be observed above and below the agar-heteroauxin discs, as well as on the petioles or at the distal and basal joints. Similar changes were never observed above or below agar discs in which no heteroauxin was present (Pl. I, Figs. 8, 9, 10–11, 12, 13).

It still remains to mention the swelling of the part of the petiole situated between the agar-heteroauxin disc and the distal joint, or between the disc and the wound which is made when the upper joint is cut off. The agar-heteroauxin disc was placed several mm. below the distal joint or beneath the wound, but occasionally this distance was increased by removal of the disc owing to growth of the petiole or because the disc had slipped from its original position. The swelling often increases in both of these circumstances to a greater extent above rather than below the agar-heteroauxin disc, and it seems at this stage that the petiole becomes more swollen when the distal joint has been removed than when it has been left intact. Subsequently, however, after passing through a stage in which the swellings on either side of the agar disc are equal, the number of swellings of this type decreases. Finally, a great majority of the petioles then show smaller swellings between the agar-heteroauxin disc and the distal joint or wound than are to be found below the disc (Pl. I, Fig. 13, *a* and *b*).

Abscission of the petioles.

After some time the petioles from which the lamina had been removed and which were consequently incapable of performing their normal function fell off (Pl. I, Fig. 8). On the other hand, none of the petioles from which the lamina had been removed became detached in this way within 19 days of the attachment of the agar-heteroauxin discs, whereas 7·7–85·7 per cent. of the petioles fell off within 11–19 days when agar-heteroauxin was not present.

Swelling of the distal joints.

Treatment.	No. of petioles treated.	Position of discs containing heteroauxin.	No. of distal joints swollen.	Percentage swollen.
A.	80	The distal joint	69	86·3
B.	36	Below the distal joint	28	77·8
C.	34	Middle of the petiole	6	17·6
D.	21	The basal joint	2	9·5

It will be seen that the percentage of distal joints which became swollen decreased through the series A–D. Later on the joint above the heteroauxin disc dried up, and only the portion below the disc remained swollen, and finally the swellings diminished in number (Pl. I, Fig. 7 *a–k*). For example, in the experiment No. 38, in which heteroauxin was applied below the distal joint, the number of petioles exhibiting swellings on a succession of days was as follows:

After 3 days 85·7 per cent.
      „   7 „   46·4 „   „
      „   10 „   35·7 „   „
      „   16 „   4·0 „   „

Meanwhile the colour of the joints changes from green to yellow and brown. Some of the joints dried up, and these remained attached to the petioles whether these were still in their normal state, or after they had dried up (Pl. I, Fig. 8).

Abscission of the distal joints.

In six experiments it was observed that of 123 untreated petioles the distal joint became detached in 73·3 to 100 per cent. within 6–13 days. On the other hand, of 123 similar petioles on 28 separate plants to which agar-heteroauxin had been attached below the upper joint only 1 had fallen off after seven days. In this same set of experiments after ten days one distal joint had dried up but still adhered to the petiole. A similar result was obtained in another experiment in which agar-heteroauxin was fixed to the distal joint in 32 plants. Here 4 joints dried up after six days and remained attached to the petiole. The number of joints which dried up without becoming detached from the petiole increased subsequently, and this seemed to be correlated with shrinkage of the petioles. A few of the joints on untreated control petioles also dried up, but all of these subsequently became detached.

When agar-heteroauxin was attached to the middle of the petioles or to their basal joints the distal joints behaved quite differently. Here also in one experiment no distal joint had fallen off within six days from the treatment of the petioles with heteroauxin, whereas of the controls 86·7 per cent. had become detached. In another experiment both types of joint still remained attached on the second day. In a test with 19 plants to which agar-heteroauxin was applied to the middle of the petiole the number which lost their upper joints was 31·6 per cent., while 94·7 per cent. with the untreated

controls. Finally, in an experiment in which the heteroauxin was applied to the basal joints of 21 plants, 52·4 per cent. fell of those which had been treated, and 80·75 per cent. of the controls.

Reaction of the basal joints.

In all of the experiments in which different methods of fixing the agar-heteroauxin discs on the petioles were tried, the basal joints could become more swollen, more elongated, and more opaque than in the controls. These changes were most pronounced when the agar-heteroauxin was attached to the lower joint (Pl. I, Figs. 10/1, 11, 12). The influence of the heteroauxin was especially well defined when the lower joints were simultaneously more opaque, longer, and thicker than those of the controls.

When agar-heteroauxin was applied immediately below the distal joint only 12·5 per cent. became opaque as well as elongated and swollen. The corresponding percentages when it was applied in the middle of the petiole were 47·4 and 66·7, while when the basal joint was treated 71·4 and 100 per cent. became opaque, elongated, and swollen simultaneously.

Agar discs without heteroauxin on the petioles.

It was also proved that if agar discs containing no heteroauxin were applied to the petioles in various positions none of the above reactions were induced, the petioles, in fact, behaving in precisely the same way as the untreated controls (Pl. I, Figs. 14, 16).

It remains to mention a further type of experiment of a somewhat provisional nature, but the results of which render the experiments which have already been described more easily comprehensible.

Bean plants, either whole, or with the epicotyls, the first internodes and petioles attached to them, were cultivated in a damp atmosphere under bell jars.

Under these circumstances the distal joints contracted or became rotten, but nevertheless remained attached to the plants when heteroauxin was present. On the other hand, when pure agar discs without heteroauxin were attached or when the petioles were left untreated the joints fell off. The maximum rotting of the petioles was noted in those which were treated with heteroauxin, those treated with agar alone were not so much affected, while those which were not treated at all were decayed least of all. These experiments also showed that, when treated with agar-heteroauxin in a moist atmosphere, the petioles became more or less equally swollen throughout their length, in much the same way as was previously found when the heteroauxin was applied in lanolin paste. This was also found to be true when the plants were cultivated under a dark cover in a greenhouse and were therefore subjected to a damper atmosphere than the other plants (Pl. I, Figs. 14–16).

DISCUSSION OF RESULTS

The curvatures of untreated petioles from which the lamina had previously been removed were generally less frequent, and also less pronounced than

those found to occur after heteroauxin in agar had been applied. Moreover, the direction of the curvatures was quite different in the presence of hetero-auxin, especially in those experiments in which the discs were attached somewhere below the distal joint. Here the maximum curvature was generally to be found in the neighbourhood of the discs, but at others above or below the discs. The treated petioles were also usually longer than the controls, especially when the heteroauxin was applied at or below the distal joint.

A striking effect in all the experiments was the swelling of the petioles. The position at which the heteroauxin was applied was unimportant in this reaction, but the location of the initial swelling was determined by the position of the disc on the petiole; the thickening generally began below the disc, seldom above it. Later on the swellings became more elongated so that a large proportion or the whole of the petiole was affected, the swelling of petiole involved being governed by the distance between the distal joint and the position in which the agar disc containing the heteroauxin was applied. The maximum number of completely swollen petioles was obtained when the agar-heteroauxin disc was attached below the wound produced by removing the distal joint, the next largest number was found when the discs were on or near the distal joint, the swelling being less frequent as the distance between the disc and the distal joint increased. The swelling was not equally well developed on the whole surface of the petiole. The largest swellings were below the disc, decreasing towards the basal or distal joint, but in the latter case, when the distance between the disc and the joint was not great, the swelling was often more pronounced above than below the disc.

These swellings subsequently decreased in size, and, after passing through a stage in which they were about equal in size both above and below the disc, a great majority of them finally ended as a smaller swelling above than below the disc. This decrease in size can be due only to shrinkage of the tissues. This shrinkage continues, but when heteroauxin has been applied on or below the distal joint the petioles remain fresh for a longer period than when the heteroauxin is applied in the middle or immediately above the basal joint. This shrinkage and desiccation of the tissues was generally more clearly to be seen in petioles to which agar-heteroauxin had been attached than in the untreated controls or in those treated with pure agar without heteroauxin; the heteroauxin also caused the colour to change to yellowish- or whitish-green. Treatment with heteroauxin also caused the petioles to remain attached to the plants for a longer period than was found in the controls. For instance, none of those treated with heteroauxin had fallen off at a time when 85·7 per cent. of the controls had become detached; in fact the heteroauxin may be described as prolonging the life of the petiole. This effect, however, was less pronounced when the agar discs were attached to the petiole well below the distal joint. It is also interesting to note that Mai (1934) found that the greater the distance between the pollinium and the basis of Coleus petioles the more likely is the petiole to remain attached to the plant.

The distal joints themselves, as distinct from the petiole, also became swollen after treatment with heteroauxin. The number exhibiting this reaction became progressively smaller the nearer the agar-heteroauxin disc was to the basal joint. If the disc was attached to the basal joint itself the distal joint became only very slightly swollen. When the agar-heteroauxin disc was attached to the distal joint shrinkage of the tissues began above the disc and subsequently proceeded downwards. The joints also turned yellowish or brown, some of them drying up and remaining attached to the petiole when the disc was fixed immediately below the upper joint. The distal joints of petioles to which heteroauxin was not applied also dried up, but they all became detached. In a few instances adventitious roots arose on the petioles, but this occurred exclusively when heteroauxin had been applied.

Most of the types of reaction caused by heteroauxin which have been described above are already well known in other plants. It is interesting to note, however, that all of them can be induced in the plant organ when the latter has lost its normal function, in this instance by removal of the lamina from the petiole. The normal function of the petiole is to join the lamina to the stem and to correlate the physiological processes of these two organs, but when the lamina has been removed it shrivels up and falls off. This shrinking and abscission of the petiole is, in all probability, primarily due to lack of water-supply caused by the loss of the upward sucking force which is normally exerted by the lamina. From what has been said above concerning the influence of heteroauxin in inducing the formation of swellings it is evident that this substance must be capable of diffusing downwards from its points of application. We may suppose that this takes place only so long as there is no considerable loss of water from the petiole by transpiration, or at least as long as any such loss of water can be made good by the uptake of fresh supplies from below. At the same time it must be borne in mind that concentrated heteroauxin also causes shrinkage and desiccation of the tissues. In spite of this effect, however, swellings were in many cases observed above the agar-heteroauxin disc. This could occur only if the water-supply increases in spite of the desiccating action of the heteroauxin. In this connexion it is interesting to mention that when the bean plants in the greenhouse were supplied with more water than usual the volume of the swellings induced by treatment with heteroauxin seemed to be increased. Also, when the plants were cultivated in a specially moist atmosphere under bell jars application of agar-heteroauxin to the distal joint induced the formation of swellings which were practically equal in diameter throughout their length. This indicates that the heteroauxin must be differently distributed in the petiole, or else that the ability of the heteroauxin to operate is increased.

It is also to be noted that heteroauxin accelerated the decay of isolated joints when kept moist in Petri dishes. The cause of this remains to be investigated, but it is known from the work of Tincker (1934, 1937) and Jacobs (1935) that growth substances can stimulate bacterial development.

As already mentioned, after removal of the lamina, abscission of the distal joints normally takes place. This process can be inhibited by the action of heteroauxin applied in agar discs, the maximum inhibition being obtained when the discs are attached on or near the distal joint. The inhibition is progressively less pronounced the greater the distance between the joint and the agar disc; in effect the heteroauxin prolongs the life of these structures. It seems obvious that this is achieved by the power of the heteroauxin to increase the supply of water to the joints, which are thereby enabled to remain fresh for a longer period. At the same time it must be remembered that in some of the experiments the joints remained attached in spite of the fact that they became desiccated. Also in the Petri-dish experiments the joints fell off unless heteroauxin was present in the agar on which they were growing, in which case the saturated atmospheric conditions within the Petri dish were not in themselves sufficient to ensure the retention of the joint. This indicates that the ability of the heteroauxin to prolong the life of the joints must be partly dependent on some cause other than that of 'drawing' water to the joint.

Heteroauxin was also found to be capable of prolonging the life of the whole petiole after the lamina had been removed. In fact, the petioles remained alive after heteroauxin had been applied in much the same way as they would have done if the lamina had not been removed. In other words, the heteroauxin seems in some respects to influence the petiole in very much the same way as the lamina. It seems reasonable to suppose that this can be achieved when the heteroauxin exercises its influence either directly by changing the distribution of the water, or indirectly by stimulating processes in the petiole, for example cell division, which need water. Mai says that the presence of pollinia in the blade-less petiole of Coleus promotes cell-divisions which prolong the life of the petiole.

Similar prolongations of life of plant organs which have lost their function have been described by several investigators. Mai (1934), La Rue (1936), and Jurišić (1937) observed similar effects in different kinds of petioles from which the lamina had been removed. Malabotti (1937), who also investigated the influence of heteroauxin in lanolin on the cotyledons of *Phaseolus vulgaris*, found that the consumption of reserve substances was not reduced, but records that the cotyledons remained attached to the plants in spite of their normal function having ceased on the consumption of the reserve substances; the petioles of the cotyledons were also greatly swollen. These results are in accord with those obtained in my own experiments. Jurišić (1939) also showed that when unripe fruits of certain of the Gesneriaceae had been cut off and hetero-auxin paste applied to the wound the life of the fruit-stalk was prolonged.

In all of these instances the organs whose abscission has been shown to be inhibited by the action of heteroauxin are normally supplied with water from below. In these cases the direction of flow of food supplies varies, but the direction of flow of water-supply is the same for all of them. Removal of the organ (e.g. lamina) to or from which the substances are being conducted normally results in the abscission of the organ through which the supplies

have hitherto been conducted. Application of heteroauxin after removal of lamina or fruit seems to be capable of restoring the normal function of conduction to the petiole or fruit-stalk, thereby prolonging the life of these organs. It is suggested that this can only occur when the flow of water through these, now functionless, organs is stimulated. *One may therefore deduce that the reactions caused by heteroauxin in concentrated solutions are the result of the control by this substance, either directly or indirectly, of the distribution and movement of water within the plant.*

SUMMARY

An extensive range of experiments has shown that o·1 per cent. heteroauxin (β-indolyl acetic acid) in agar is capable of inducing and stimulating curvatures, elongations, and various types of swelling in the petioles of *Phaseolus vulgaris* from which the lamina had been removed. These changes occur in the petiole itself as well as in the joints which are situated at the base of the petiole and just below the point of attachment of the lamina to the petiole. These joints are referred to as the basal and distal joints respectively. Heteroauxin is also capable of inhibiting the abscission of the distal joint, or of the whole of the petiole at the basal joint, if applied in appropriate positions after removal of the lamina. The heteroauxin may be said in effect to prolong the life of the petioles which have lost their normal function through removal of the lamina. The view is put forward that the heteroauxin in concentrated solutions operates by controlling, either directly or indirectly, the distribution and movement of water within the plant. In the latter case it may operate by changing the conditions in the petioles or stalks.

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EXPLANATION OF PLATE I

Illustrating Dr. L. Portheim's article on 'Further Studies on the Action of Heteroauxin on *Phaseolus vulgaris*'.

All figures show the reaction of petioles of *Phaseolus vulgaris* with the lamina detached.

Fig. 1. Petioles treated with lanolin-heteroauxin paste on the distal joints; club-shaped swellings on the petioles; petioles bent.

Fig. 2. Petioles treated with lanolin-heteroauxin paste on the basal joint; club-shaped swellings on the petioles.

Fig. 3. Plants cultivated in a damp atmosphere. Petioles treated with lanolin-heteroauxin paste on the distal joint; more equal swelling on the whole petiole.

Fig. 4. Petioles treated with lanolin alone on the distal joint in a damp atmosphere.

Fig. 5. *a*, and *b*. Untreated petioles.

Fig. 6. *a*, and *b*. Petioles with agar-heteroauxin discs on the distal joints; swelling of the distal joints and of the petiole-parts below the distal joint; petioles bent upwards.

Fig. 7. Petioles treated with agar-heteroauxin discs on the distal joint. Enlarged. *a-b* Controls, untreated. *c-f*. Swelling of the distal joints and of the parts of the petioles below the distal joint. *g-k*. The later decrease in size of the swelling and the drying up of the distal joints is visible.

Fig. 8. Petiole with agar-heteroauxin disc in the middle of the petiole. On the left: treated petiole; swelling below and above the disc; the greater part of petiole above the disc drying up; the distal joint dried up but still adhering. The untreated petiole on the right has fallen.

Fig. 9. Petiole with agar-heteroauxin disc in the middle of the petiole. On the left: treated petiole; swelling of the petiole below the disc and of a part of the petiole above the disc; the apex of the petiole drying up; the dried up distal joint falling off. On the right: untreated petiole, with distal joint fallen.

Fig. 10. *a*. On the left: petiole with agar-heteroauxin disc above the basal joint; whole of the petiole as well as the distal and the basal joint swollen. On the right: untreated petiole; the distal joint dried up. *b*. Petiole with agar-heteroauxin disc in the middle. The whole petiole and the basal joint swollen; the distal joint present. On the right: untreated petiole; the distal joint fallen.

Fig. 11. Petiole with agar-heteroauxin disc above the basal joint which is swollen; the distal joint fallen. On the right: untreated petiole with the distal joint fallen.

Fig. 12. Petiole with agar-heteroauxin above the basal joint. The whole petiole swollen especially between the disc and the basal joint. The distal joint present; petiole bent. On the right: untreated petiole with distal joint fallen.

FIG. 13. Petioles treated with agar-heteroauxin below the distal joint. Swelling on the part of the petiole between the distal joint and the disc. *a*. On the left: untreated petiole. The distal joint fallen. On the right: treated petiole. The parts of the petiole below and above the disc equally swollen; the distal joint present, the basal joint swollen. *b*. On the left: untreated petiole. The distal joint fallen. On the right: treated petiole. The whole petiole swollen; but the part above the disc less swollen; the distal joint present.

Figs. 14–16. Petioles of plants in a dark room. Fig. 14. Controls, the distal joints falling off. Fig. 15. Petioles with agar-discs on the distal joints; one distal joint fallen. Fig. 16. *a*, and *b*. Petioles treated with agar-heteroauxin discs on the distal joint. The left-hand petioles in *a* and *b* are equally swollen throughout their length after being treated.
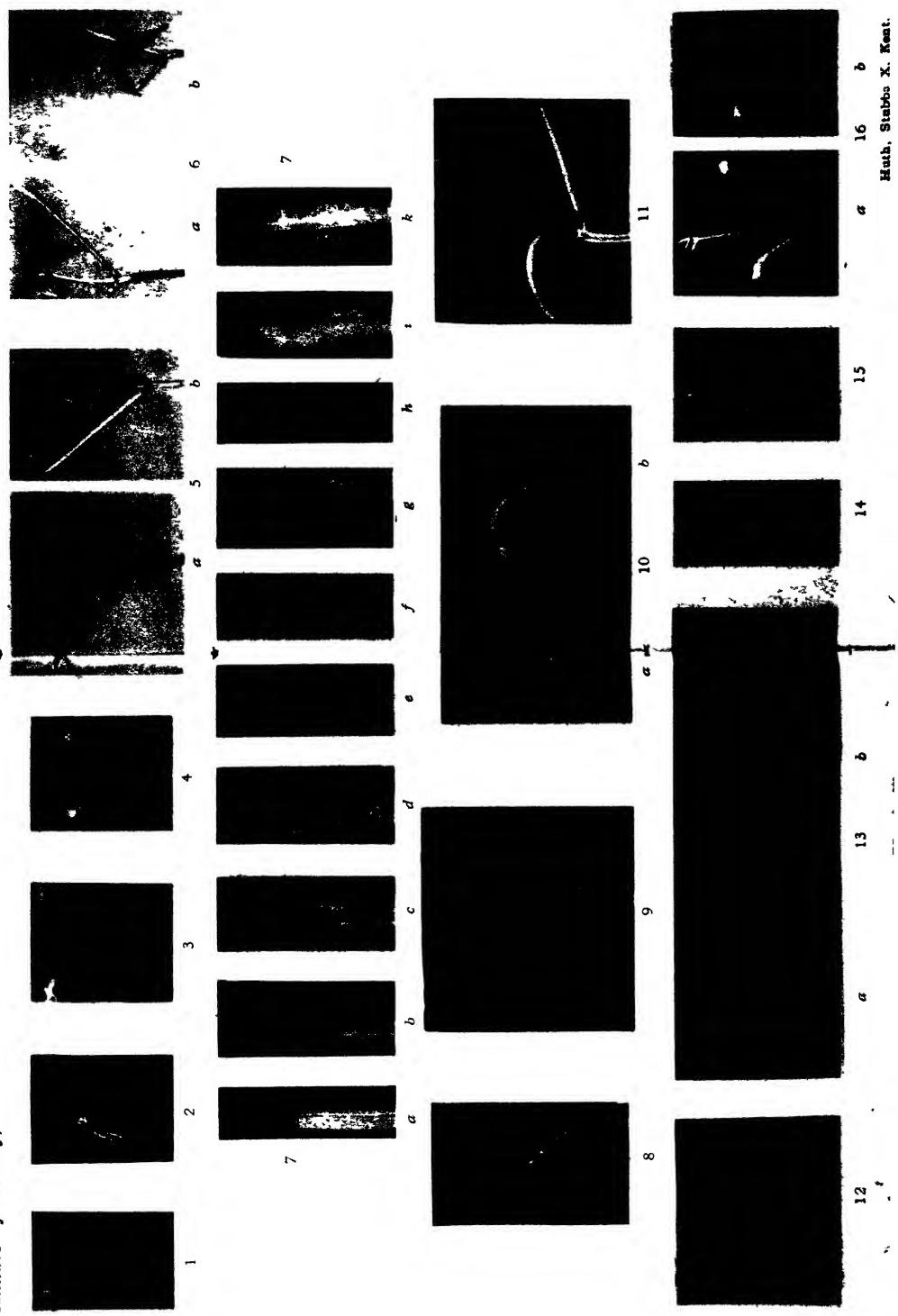
Caytonanthus, the Microsporophyll of Caytonia

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With Plate II and eight figures in the Text

THIS paper describes Caytonanthus, delimits the species, relates them to their appropriate leaves and fruits, and brings up to date the evidence for referring the various detached organs to the Caytoniales. The material was collected by Mr. F. M. Wonnacott from Gristhorpe Bay, Yorkshire, for the Geological Dept. of the British Museum, and is of Middle Estuarine (Bajocian) Age.

Genus Caytonanthus Harris

1925 *Antholithus* Thomas, p. 327 (figures, description, and restoration).
1937 *Caytonanthus* Harris, p. 40 (figures, diagnosis, and restoration).
For other references see under *C. arberi* and *C. thomasi* below.

Thomas gave the first useful account of a Caytonanthus species under the name *Antholithus arberi*, the name Antholithus being a non-committal designation for reproductive organs rather than a genus. Caytonanthus was instituted as an organ-genus when a second species was described; a third species is described here, but all are closely similar except in details. In the following description Caytonanthus is termed a microsporophyll, consisting of a pinnately branched rachis, the lateral branches dividing into ultimate branchlets each bearing an anther-like synangium composed of four pollen-sacs.

Description of the Specimens

Caytonanthus is represented in this collection by three microsporophyll fragments; some isolated anthers; a large number of isolated pollen grains and pollen grains in the micropyles of pollinated seeds.

1. *The microsporophylls.* All three specimens, after being photographed, were made into balsam transfers.

Specimen V 18595 (Pl. II, Figs. 9, 10) which belongs to *C. oncodes* was collected and presented by H. H. Thomas. It consists of the upper part of a microsporophyll of which the apical 2 mm. has been lost, though still represented by a faint print in the matrix. On transferring the specimen was little altered in appearance, most of the synangia prove to have been lost before preservation leaving distinct scars, but two are still attached. The most

striking feature of this specimen is its flatness and the comparative regularity of its branching.

Specimen V 25897, which is the type-specimen of *C. oncodes* (Pl. II, Figs. 5, 8, 11), showed only a few synangia until transferred, when the rachis and many more synangia were exposed. Nearly all the branchlets still bear their synangia, but these are ripe and have shed their pollen. A striking feature is that although the branches are opposite as in Thomas' material they are unequal, that on the right of the transfer bearing two and that on the left at least eight synangia, some of which are, however, of small size. Each synangium is borne singly on an ultimate branchlet, which it appears to terminate, but comparison with V 18595 shows that the synangia were attached on one surface, just short of the apex.

The third specimen (V 25903, Pl. II, Figs. 3, 4) is *C. arberi*; it shows a rather thick rachis. It came adrift from the balsam in transferring, but the rachis and synangia were recovered. This specimen again shows the upper part of the microsporophyll, and as in V 25897 the apex dichotomizes. The rachis had suffered twisting through nearly 180° in preservation, but allowing for this all the branches again lie in the horizontal plane. The branches are short, and their ultimate branchlets so small as to form mere lobes, so that when all the synangia were attached they must have appeared to form tufts, but separate attachment scars can be recognized here also, just as in the other species.

The rachis was cleared by the action of chloric acid diluted with acetic acid, when it gradually became translucent and showed a dark central strand from which side branches emerged at an acute angle; individual vascular bundles were not seen. A feature common to this and the other specimens is that the surface of the rachis lacks the distinct ribs seen on the rachis of Caytonia and the stem of Sagenopteris, and was presumably a less rigid organ.

The isolated synangia consist of some which came adrift in transferring the above specimens and most probably were attached to them, also one separate specimen which belongs to *C. arberi*. The majority are quadrilocular, one proved to be trilocular (Pl. II, Fig. 1), and another bilocular with pollen sacs placed opposite. Their form in their compressed state is fully consistent with the symmetrical, four-winged form shown in Thomas' restoration. The dehisced sporangia show clearly the method of dehiscence; the four pollen sacs while remaining attached at base and apex drew away from one another in the middle leaving four distinct and symmetrical gaps, and then opened individually along their inner margins. Not infrequently the pollen sacs have come apart at the apex too, as seen in Pl. II, Fig. 1, and in Thomas' figure (1925, Fig. 36*a*). In these dehisced synangia there is no central core of tissue corresponding to a connective; there is not even a suggestion of a central vascular strand. It will be seen, therefore, that Harris (1937, p. 40) was incorrect in inferring from the existence of a gap in the middle of sections of a macerated unripe specimen that a substantial core was situated here. The outer wall of the pollen sac is dense and opaque; when partially cleared it shows elongated thick-walled cells

but no sign of walls with more delicate claw-like thickenings such as are seen in flowering-plant anthers.

The cuticle of the rachis of V 25903 was prepared; it proved to be of moderate thickness, appearing to be only a little more delicate than that of Sagenopteris leaf petioles. The dorsiventral structure described by Thomas was confirmed. One side (Text-fig. 1) looks about twice as thick as the other; in the former the longitudinal cell walls are better marked than the transverse, in the latter all walls are of even thickness. A very few small trichome bases were observed on the thinner side. It was hoped that it would be possible to trace the relation of the side bearing synangia to the thick and thin sides, and so to show by comparison with the structure of the leaf and fruit rachis which way the synangia faced. The preservation was not, however, adequate for a decision. The minute portions of cuticle prepared from the rachis of the other specimens showed similar structure.

The synangium wall has a rather thin cuticle, thinner than that of the rachis, but by no means so thin as to be difficult to prepare and mount. In all specimens examined the cuticle shows uniform elongated hexagonal cells over the greater part of the wall which become narrower towards the inner margins. At the apex, in some specimens at least, cutinized unicellular hairs occur. Besides the outer cuticle, most specimens provide vestiges of an inner obscurely cellular granular membrane to which the pollen grains often adhere; perhaps this membrane is the fatty matter of the tapetum hardened in preservation.

The pollen grains are represented by their cuticles alone, and the two surfaces are flattened together but often show very little other distortion. The wings are usually placed symmetrically at the sides of the central cell, but in a good many which showed no other signs of distortion they are nearer one side. In many of the grains in the micropyles of seeds the central cell has burst, but I hesitate to attribute this to germination, although I gained the impression that the proportion of burst grains in seeds is higher than in anthers or in loose grains in the rock matrix.

Discussion of Morphology

The present specimens by their leaf-like branching and dorsiventral structure fully confirm Thomas' view of the whole fossil as a microsporophyll. The attachment of the synangia to the surface, and above all their structure and dehiscence, brings them into relation with the synangia of fossil Marattiales and Pteridosperms; it makes them very different indeed from any flowering-plant anther where the presence of a vascular connective and a basic bilateral symmetry appear to be fundamental.

A fact, not previously pointed out, is that, Caytonanthus, though by no means a robust fossil is not specially delicate. It is far denser in substance than the associated conifer male cones and is of similar density to the leaf Sagenopteris; its cuticle though neither thick nor tough is only a little thinner
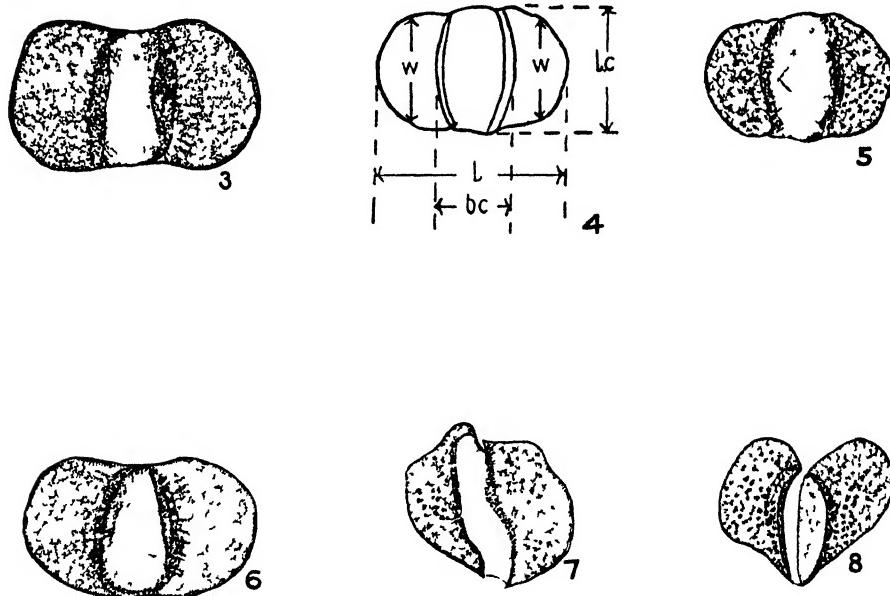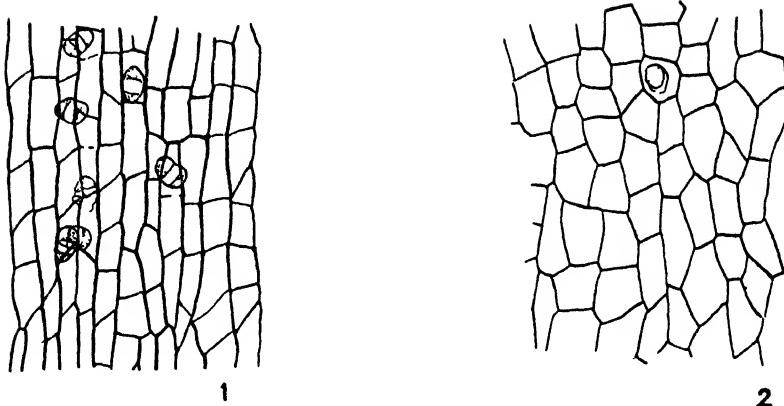
TEXT-FIGS. 1–8. Fig. 1. *Caytonanthus arberi*, cuticle of rachis (thicker side) with a few pollen grains; V 25903. ×20. Fig. 2. Thinner side of same rachis with a trichome. ×200. Fig. 3. *Caytonanthus oncodes*, unusually large pollen grain from synangium, V 18595 (*f*). ×1,000 Fig. 4. Outline of *C. arberi* pollen grain showing where dimensions are measured; *l.* length from wing to wing, *b.c.* breadth of central cell; *l.c.* length of central cell; *w.* width of wing measured at its middle. Fig. 5. *C. arberi* pollen grain from synangium, V 25903 (*k*). Fig. 6. *C. oncodes* pollen grain from micropyle of *Caytonia sewardi* seed, V 26654. × 1,000. Fig. 7. *C. arberi*, large pollen grain from micropyle of *Caytonia nathorsti* seed, V 26724. × 1,000. Fig. 8. *C. arberi*, small pollen grain from micropyle of *C. nathorsti* seed, V 26722. × 1,000.

than that of a Sagenopteris leaf. In these features it differs from such few flowering-plant stamens as I have examined, which as would be expected possess far less wall material and a much thinner cuticle than the corresponding leaf. Fossils agreeing in this respect with Caytonanthus are Antevsia, the microsporophyll of Lepidopteris (see Harris, 1932, p. 62) and Hydropterangium, the microsporophyll of Ptilozamites (see Harris, 1932a, p. 122) both Mesozoic plants of Pteridosperm affinity. The inference put forward is that Caytonanthus was a fairly long-lived structure produced in an exposed position like a leaf, not an evanescent structure developing almost to maturity in shelter like a stamen.

Winged pollen like that of Caytonanthus is unknown in flowering plants, but is known in the microsporophyll of Ptilozamites (mentioned just above), while its very wide occurrence in Mesozoic rocks suggests that many other plants produced similar grains.

These points emphasize the connexion of Caytonanthus with the Pteridosperms, while sundering its connexion with the flowering plants.

Description of Species

Caytonanthus arberi (Thomas) Harris

Selected type-specimen, Thomas, 1925, Pl. 14, Fig. 33. The following specimens may belong to this species or to *C. oncodes*:

1875 'unknown leaves' Phillips (Pl. 7, Fig. 23).

1900 *Gingko digitata* flowers, Seward (p. 259; Text-fig. 45).

1919 *Antholithus sp.* Seward (p. 51, Text-fig. 654 A, B).

1920 *Ginkgoanthus phillipsii* Johnson (p. 1, Fig. 1–3, 6, 8).

1925 *Antholithus arberi* Thomas, p. 327 (Pl. 14, Figs. 33–40, 42.) (The specimens shown in Figs. 34–5, 38, 40, 42, have definite points of agreement with *C. arberi.*)

1931 *Antholithus arberi* Thomas (p. 651, discussion).

1937 *Caytonanthus arberi* (Thomas) Harris (p. 44). Name; possibly specimens figured in Text-fig. 4 A–C as *C. sp. A* (but not specimens described and figured as *C. arberi*).

Emended diagnosis. Microsporophyll rachis bearing short lateral branches, lateral branches lobed but scarcely subdivided, synangia typically about 3 mm. × 1 mm.; pollen grains typically 22 μ from wing to wing (extremes 18—28 μ), central cell about 15 μ long; wings seldom bulging but often slightly constricted; mean width of wing 13·5 μ (extremes 9—18 μ), surface of wings pitted, but pitting not very conspicuous. For comparison see below.

While it is impossible without a re-examination of their pollen grains to state how many of Thomas' original specimens belong to this species, it appears that the pollen grains he figures agree with *C. arberi* as interpreted here, while with the type-specimen (Fig. 33) the present specimen agrees in the shape and attachment of the synangia on condensed lateral branch

systems. The fragments shown in his Text-fig. 9 A, on the other hand, appear more like *C. oncodes*.

Caytonanthus oncodes sp. nov.

? 1937 *Caytonanthus arberi* (non Thomas); Harris, p. 44, Text-fig. 4 D–G. (Figures of isolated pollen grains.)

Diagnosis. Microsporophyll rachis bearing fairly long lateral branches which subdivide once or twice into finger-like branchlets. Synangia typically broad, about 2·5 mm. × 1·5 mm. Pollen grains typically 31 μ from wing to wing (extremes 25—35 μ), central cell about 17 μ from end to end, wings bulging slightly, seldom contracted, mean width 16·5 μ (extremes 10—22 μ). Surface of wings clearly and rather regularly pitted.

Type-specimen, V 25897, Pl. II, Figs. 5, 8, 11.

For comparison, see below. The name refers to the bulging form of the wings.

For diagnosis, description, and figures of *Caytonanthus kochi* Harris, see Harris, 1937, p. 43, where other references are given.

Comparison. There is reason to suppose that the microsporophyll is of slightly different form in the three species, but this view is based on a very few specimens. In *C. kochi*, where the rachis is unknown, the lateral branchlets are particularly slender and the synangia are usually a little longer than in the other species; in *C. oncodes* the lateral branches are much divided and the synangia are particularly short and wide; in *C. arberi* the lateral branchlets are very short and scarcely divided, the synangia being intermediate in size.

The pollen grains at present provide the only certain means of distinguishing the species. *C. Kochi* has the largest grain with almost smooth wings, *C. oncodes* a grain of intermediate size with strongly pitted wings, and *C. arberi* the smallest grain with rather obscure pitting.

The separation of the species of Caytonanthus.

In Thomas' original account of the Caytoniales there is a gap caused by there being only one microsporophyll for two types of fruit. Clear evidence for the existence of two types of pollen was in the present investigation first obtained among the grains in the micropyles of the seeds; the two types were then traced to their respective microsporophylls. While the species may probably differ in the gross form of the microsporophyll, it is the differences of dimensions of the pollen grains which must at present be used (see p. 53) and considerable difficulty is caused by the great overlap in the range of size variation; thus the largest in a synangium of *C. arberi* though larger, of course, than the smallest of *C. oncodes*, would even pass for unusually large grains of that species. This difficulty, which is increased by the effects of distortion caused by crushing, was overcome by the use of statistical methods which so far as I know have not previously been used in separating fossil plant species.

What appeared to be the least distorted dimension was selected for closer study. This is the width of the wing, measured transversely through its middle (Text-fig. 4 D). The spores were measured by drawing with a camera lucida, at high magnification, repeated trials with a single specimen having shown that when precautions were taken the error was within $\pm$ 0·5 μ. All available spores of each specimen were measured, and where possible both wings of each spore were measured and entered independently into the statistics, a procedure which appeared better than measuring only one or taking a mean. The means and standard deviations of each set were then calculated in the usual way. It was found that the data accorded very well with a normal frequency of error curve, and this was confirmed by using the χ^2 test.

In all, six lots of spores were examined—two microsporophylls of *C. arberi*, two microsporophylls of *C. oncodes*, the pollen in micropyles of seeds of *Caytonia nathorsti*, and the pollen in micropyles of *Caytonia sewardi*. These six lots divide clearly, as the results show, into two groups.

TABLE I

Specimen	Number of wings measured	Mean width (μ)	Standard deviation (μ)
V 26714	32	13·9	2·41
V 25903	158 ·	13·3	2·16
Caytonia nathorsti . . .	71	13·6	2·11
22 seeds			
V 25897	19	16·7	2·36
V 18595	151	16·8	1·70
Caytonia sewardi . . .	69	16·85	2·75
18 seeds			

The first three are determined as *C. arberi*, the second three as *C. oncodes*.

By applying to these figures Fisher's '*t*' test it can be seen that the three means of the first set are not significantly different from one another, and the same is true for the means of the second set, but the difference between any one of the first set and any of the second set is highly significant. That is to say, the probability against the difference of means between the most divergent of the first set (i.e. V 26714 and V 25903), being due to sampling, is only about 3 : 1; while the chance against the difference between the least different of the two sets, namely V 26714 and V 25897, being due to sampling, is more than 1000 : 1. Similarly the probability that the means of V 25903 and V 18595 owe their difference to sampling is less than 1 in a million or practically nil.

The sizes of the pollen grains of these two sets are thus really different.

Of the possible explanations of the difference the one which is here adopted is that the spores were naturally different, having indeed come from plants of different species, but another possible explanation would be that the spores were originally alike, the differences being caused in preservation and preparation. Differences due to preservation can probably be ruled out in these

fossils preserved in the same bed, but differences due to difference of treatment are possible enough, as it is known that maceration if very long continued causes swelling of cutinized spores. In practice the specimens were macerated for just as long as seemed necessary for each rather than for a standard time; the denser the specimens the longer the time. The mixture of $HNO_3 + KClO_3$ used could not be standardized as its composition changes as maceration proceeds.

Many different pieces of each specimen were macerated separately to investigate this possibility. The pollen of each piece as measured and the mean and standard deviation were found for each of these pieces. The grains from one specimen would all have been similar before treatment, and if differences in the maceration were the cause of these differences of size some of these lots of pollen grains should appear much larger than others. Table II below gives the data, all preparations providing ten or more measurements being included.

TABLE II

Preparation No.				Number measured	Mean (μ)	Standard error of mean (μ)
V 25903 (b)	.	.	.	10	12·8	0·77
V 25903 (d)	.	.	.	13	12·0	0·69
V 25903 (f)	.	.	.	16	14·0	0·32
V 25903 (g)	.	.	.	61	13·6	0·30
V 25903 (h)	.	.	.	17	13·2	0·45
V 25903 (i)	.	.	.	14	14·1	0·33
V 26714	.	.	.	32	13·9	0·43
V 18595 (b)	.	.	.	16	16·3	0·33
V 18595 (c)	.	.	.	92	16·8	0·20
V 18595 (e)	.	.	.	20	16·9	0·39
V 18595 (f)	.	.	.	23	17·0	0·49
V 25897 (d)	.	.	.	10	16·5	0·25

For V 25903, which is *C. arberi*, the total number counted was 158, the common mean 13·3 μ, and the standard error of the common mean 0·18 μ.

For V 26714, which is *C. arberi*, the above data are complete as this is the only piece macerated.

For V 18595, which is *C. oncodes*, the total number counted is 151, the common mean 16·8 μ, standard error 0·14 μ.

For V 25897, which is *C. oncodes*, the total number counted is 19, common mean 16·7 μ; standard error 0·54 μ.

The remaining preparations which provide fewer than ten measurements give means which are not, taken by themselves, of such significance, but it so happens that every one of these means for *C. oncodes* is higher than every mean for *C. arberi*.

The means given in Table II fluctuate more than the common means (given above and in Table I) because the numbers concerned are smaller. The fluctuations of mean can, however, be shown to be no greater than is to be expected when the known variability of each species is taken into account.

None of the means in Table II differ significantly from the common mean for the species.

Pollen in seeds. The pollen grains in seeds usually occur at the very base of the micropyle in the gap at the top of the nucellus cuticle. Here it is often rather difficult to see them distinctly because they are enclosed in several layers of cutinized cells, and it is fairly certain that a good many pollen grains must have been missed altogether. Difficulty was experienced at first in obtaining preparations with intact micropyles, because the micropyle which is the only thing anchoring the nucellus cuticle to the integument cuticle is easily torn and lost, and in this way perhaps four-fifths of the available seeds were sacrificed before the difficulty was overcome by finishing the maceration of each seed separately in a minute drop of ammonia on a slide. It then became possible to demonstrate pollen in a good proportion of seeds; of the last batch macerated nearly a third showed pollen, a third while well preserved showed none, and about a third were poorly preserved or spoilt in preparation. The total number of seed preparations showing pollen is 22 for *C. nathorsti*, 18 for *C. sewardi*, but as most of these have several grains the number of pollen grains seen in seeds is considerable. Pollen occurs equally in isolated seeds and seeds from intact fruits, though these latter are less easy to prepare.

It is almost certain that these pollen grains entered by the normal pollination mechanism, and not passively in the early stages of preservation, or in the preparation of the seed by maceration. There is evidence that the micropyle of the ripe seed is closed at the time of preservation, for while there may be plenty of fine mineral matter in its mouth, none has penetrated into the canal of the micropyle, as must have happened if the mud with pollen, in which the seed was buried had entered. It is impossible that the pollen entered in preparation as the micropylar canal has flattened entirely, the two cutinized sides being everywhere adherent to one another.

Without exception these grains appear to be of the Caytonanthus type; most of them show their form clearly, and even those which are too distorted or aggregated into masses to show their shape still show the characteristic fine pitting of the cuticle of the wings.

The reference of separate organs to the Caytoniales.

The organs concerned are shown below:

Age				Megasporophyll	Microsporophyll	Leaf
Basal Lias	.	.	.	*Caytonia thomasi*	*Caytonanthus kochi*	*Sagenopteris nilssoniana*
Lower Oolite	.	.	.	*Caytonia nathorsti*	*Caytonanthus arberi*	*Sagenopteris phillipsi*
Lower Oolite	.	.	.	*Caytonia sewardi*	*Caytonanthus oncodes*	*Sagenopteris colpodes*

Besides these there are many species of Sagenopteris leaves, two of which,

S. hallei and *S. dentata* (see Harris, 1932*a*), have been found associated with Caytonanthus-like pollen.

The evidence is (1) association in the field, (2) pollination, and (3) agreement of structure.

It is unnecessary to go over the association evidence fully as it is expressed as fully as possible in the papers of Thomas (1925 and 1931) and Harris (1933, p. 104). It is sufficient to say that the reproductive organs are found together, and only in those few localities where the leaf is abundant. They occur together in three countries (Greenland, England, and Sardinia) and in rocks of two ages (Lower Lias and Lower Oolite). It would be worth re-investigating the occurrence in Yorkshire in view of the present fuller knowledge of the two species concerned.

Evidence of pollination. Pollination supplies evidence of association between male and female reproductive organs of a very intimate kind. It is recognized that pollen of other plants may often reach stigmas or ovules, cf. Sahni's (1915) observation of various types of foreign pollen in Botanic Garden material of Ginkgo; see also Oliver (1915); but it will be agreed that where many specimens all show the same type of pollen to the exclusion of others, then there is a strong reason for presuming that the pollen belongs to this plant; and where three different species of seeds each shows its own species of the same general type of pollen, and further where the species concerned come from different lands and rocks of different ages, the evidence is very greatly strengthened.

The occurrence of nothing but the Caytonanthus pollen grains in these seeds is striking enough, and is the more remarkable because it is by no means the commonest type of grain in the Caytonia-bearing layers. Thus one sample when counted gave the following results: of 1,218 spores and pollen grains examined, only 10 belonged to Caytonanthus, scarcely 1 per cent. It is thus clear that a rather precise pollination mechanism operated; this has been discussed in a previous paper (Harris, 1940*a*).

It is worthy of note that Caytonanthus pollen grains have been found in large numbers sticking to the upper cuticles of Sagenopteris leaves where they far outnumber other kinds of spores. One preparation of a Greenland *S. nilssoniana* leaf shows a very high concentration—varying between 300 and 1,500 pollen grains per sq. mm.; such grains must have fallen on to the leaf in life and prove that this Caytonia flowered at a season when the leaves were out. On the other hand, none of my preparations of the Yorkshire species *S. colpodes* and *S. phillipsi* show any Caytonanthus pollen; no conclusion should be drawn from this absence from a few leaves, but it suggests a line of work worth following.

Evidence from agreement in structure.

Thomas (1925) showed that there was general agreement between the epidermal structure of the petiole of *Sagenopteris phillipsi*, the fruiting rachis of

Caytonia sewardi and *C. nathorsti*, and the microsporophyll rachis of *Caytonanthus arberi*. The evidence was, however, obscured by the confusion of two species under the name *Sagenopteris phillipsi*, so that only points of generic, rather than specific, agreement could be recognized. Now, however, the two leaf species are separated (Harris, 1940) and each can be shown to have very close agreement in cellular details to a fruit (Harris, 1940a); the agreement being most striking between the upper epidermal cells of the leaf and the epidermis of the immature fruit (the great thickening of the ripe fruit obscures the agreement). The special character of *S. phillipsi sensu stricto* and *Caytonia nathorsti* is that the cell walls are thick and straight, enclosing a sculptured cell surface; that of *S. colpodes* and *C. sewardi* thin, very sinuous cell walls. In the Greenland species *S. nilssoniana* and *C. thomasi*, the cell walls are thin and straight, and both petiole and fruiting rachis bear peculiar glandular hairs.

No fresh evidence is forthcoming for the microsporophyll: that of *C. arberi*, the only species adequately known, shows very close agreement with *Caytonia nathorsti* and *S. phillipsi sensu stricto*.

All this evidence is circumstantial and not of a nature to provide rigid proof of connexion, but such evidence can when cumulative be so overwhelming as to carry conviction.

In conclusion I would like to express my gratitude to the Trustees of the British Museum for entrusting their material to me for examination and particularly to Mr. F. M. Wonnacott and to the Keeper of Geology, Mr. W. N. Edwards, for valuable help.

Summary

1. Caytonanthus, the microsporophyll of Caytonia, is reinvestigated; *C. oncodes* sp. nov. is described.

2. *Caytonanthus oncodes* is referred to *Caytonia sewardi* which it pollinates; *C. arberi* is referred to *Caytonia nathorsti* which it pollinates.

3. Caytonanthus is shown to be a moderately robust organ and it is suggested that it was produced in an exposed position such as would be occupied by a leaf, not by a stamen in a flower.

4. The synangium of Caytonanthus differs greatly in construction and mode of dehiscence from the anther of a flowering plant, and provides strong evidence for associating the Caytoniales with Pteridosperms rather than with the Angiosperms.

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EXPLANATION OF PLATE II.

Illustrating Professor T. M. Harris's article on ' Caytonanthus, the Microsporophyll of Caytonia '

Fig. 1. *Caytonanthus* sp., detached synangium composed of three sporangia; the top one is broken at its middle, the left one shows the line of dehiscence. No. V 26717. (× 10.)

Fig. 2. *C. arberi*, sporangium dissected out from a dehisced synangium, showing the aperture. No. V 26715. (× 10.)

Fig. 3. *C. arberi*, microsporophyll axis isolated by HF; the specimen is twisted at its middle. No. V 25903. (× 4.)

Fig. 4. *C. arberi*, same specimen as in Fig. 3, before treatment with HF. The fossil to the right is a conifer male cone. Photographed under xylol. No. V 25903. (× 4.)

Fig. 5. *C. oncodes*, type-specimen before transferring, photographed under xylol. No. V 25897. (× 4.)

Fig. 6. *C. oncodes*, synangium, possibly detached from type-specimen, showing gaps between the sporangia. No. V 26718. (× 8.)

Fig. 7. *C. oncodes*, synangium possibly detached from type-specimen showing the small attachment point. No. V 26719. (× 8.)

Fig. 8. *C. oncodes*, type-specimen after transferring. No. V 25897. (× 4.)

Figs. 9, 10. *C. oncodes*. Fig. 9, under xylol; Fig. 10, in transfer, showing the branches all lying in the horizontal plane. No. V 18595. (× 4.)

Fig. 11. *C. oncodes*, part of the type-specimen, showing how the synangia are attached. In the upper synangia there is a narrow gap running between the upper and lower sporangia. (× 8.)

Figures 3–11 are from untouched photographs by Mr. L. C. Willis.

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HARRIS — CAYTONANTHUS.

Studies of the Physiology of Coffea arabica

III. Transpiration Rates of Whole Trees in Relation to Natural Environmental Conditions

BY

F. J. NUTMAN

(East African Agricultural Research Station, Amani, Tanganyika Territory)

With nine Figures in the Text

INTRODUCTION

WHEN Balls (1937) described a method of weighing by the use of an automatic chain balance[1] a valuable instrument was made available to the plant physiologist. It has always been recognized that the weighing of rooted plants grown in sealed containers is the only sound method for determining transpiration rates. But, especially where large plants are used, the weight of the plant and its container is very great compared with the weight-changes to be measured, and large balances are seldom sensitive. Consequently measurement of weight-loss over short time-intervals has been impossible.

Balls claimed that his method enables weighings to be made on a steelyard with an accuracy approaching that of a good chemical balance, and quotes data illustrating an accuracy of 1 : 350,000. I have used a modification of his method and have found that his claims can readily be confirmed and are in fact conservative. Thus it becomes possible to record the march of the transpiration rate of large plants over very short time-intervals, and the relation between the march of transpiration rates and the varying meteorological factors can be studied in a way hitherto impossible.

This paper reports the results of such studies. Further work had been planned as a result of the data here presented, and publication would normally have been delayed in order that additional data could be included. The outbreak of war, however, resulted in the temporary interruption of this work, and consequently this paper is not as comprehensive as might be wished.

THE SITE OF THE EXPERIMENTS

This work was carried out on a coffee estate at Arusha, in Tanganyika Territory, at an altitude of about 4,300 ft. Coffee is here grown at nearly

[1] This type of balance is sometimes described as 'chainomatic'. This meaningless word appears, most regrettably, to have become established, at least in manufacturers' catalogues.

the limit of its climatic range, the conditions, as can be seen from the climatic data presented, being sometimes extreme. Nevertheless production in this district is considerable.

Two stations were chosen for the work. The first was a completely exposed site, totally unshaded, the ground cover being short grass. A wind-break of hessian cloth was erected some little distance from the apparatus and material. This was far enough away for its shade not to affect the trees under study. The almost constant direction of the wind enabled a short wind-break to be effective. The second station was inside a coffee plantation, also unshaded. The trees were spaced 9 ft. by 9 ft., and those adjacent to the experimental material proved to be adequate wind-breaks: as a result the experimental trees were exposed to the wind normally present inside the plantation.

It would have been desirable to include a third station, in a plantation provided with natural shade, but this was not possible. The effects of a reduction in light intensity without at the same time altering other climatic factors were studied by arranging a small screen of hessian cloth between the experimental tree and the sun. This reduced the radiation by about 70 per cent. This shade, of course, is in no way equivalent to natural shade, for the meteorological data obtained under it (excepting total solar radiation) did not differ from that obtained outside, doubtless because of its extremely small area.

MATERIAL

All the work here described was carried out on young trees of *Coffea arabica* grown at Amani in metal containers holding about 450 lb. of soil. The trees were about 5 ft. in height and had an average of about 1,100 leaves. They were transported more than 300 miles to the site of the experiments by road and rail, great care being taken to ensure that the soil in the containers remained undisturbed throughout the journey. Thermo-hygrograph records showed that the precautions taken to ensure that the van in which they travelled remained moist and cool were effective.

The plants appeared unaffected by the journey and growth continued unchecked. As a precaution against any possible ill effect, however, transpiration measurements were deferred until after several weeks.

APPARATUS AND METHODS

My arrangements were very similar to those of Balls, and his original paper should be consulted.

The tree under investigation was suspended from a steelyard hung from a large metal tripod. The long arm of the steelyard carried an electrical contact, and one end of the hanging chain was attached to it. I found it necessary to enclose both the steelyard itself and the hanging chain, for they are affected by wind and by insects settling on them. The following description of parts of the apparatus is mainly confined to the differences between my own apparatus and that of Balls.

1. *The reversing gear.* This gear, illustrated diagrammatically in Fig. 1, differs from Balls' in having only one train of epicyclic gears and in having the pawl and pinion external to the gear-case. The pawl itself forms one diameter of a large ring which completely encircles the gear-case and which
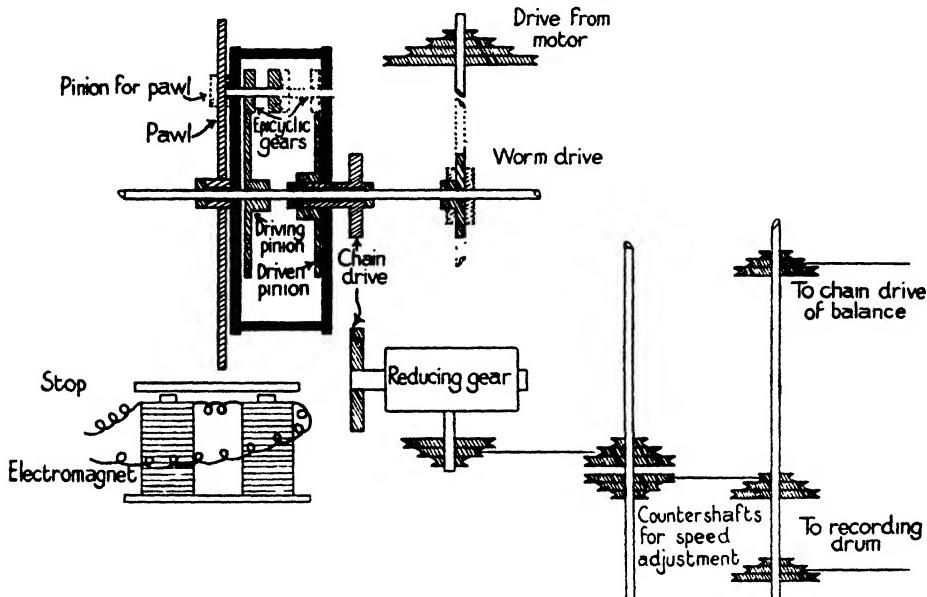
FIG. 1. Diagram of reversing gear and connexions.

carries twenty-five projections, these engaging with the electromagnetically operated stop. Consequently, reversal follows immediately on the operation of the stop, and the gear can be run at very low speeds without loss of efficiency from delayed reversal.

The drive from the gear is taken by chain to a 30 : 1 reduction gear, and thence to a system of countershafts with variable pulley drives, providing a wide range of final speeds. The gear-box normally operates at about 50 revolutions per minute.

2. *The steelyard assembly.* Fig. 2 illustrates the arrangement finally adopted. The chain is hung from a knife-edge, resting in a V notch filed in an extension of the long arm of the steelyard. This notch is in the same plane as the four knife-edges of the steelyard. Two strands of Meccano chain have proved sufficient for the recording of twelve hours' transpiration. The effective length of this chain is controlled by the reversing mechanism as described by Balls.

Balls used a metal-to-metal electric contact, the oscillation of the beam being limited by stops. I have found this method unsuitable, at any rate out of doors, because of 'bounce' at the contacts induced by air movements. Under such conditions I have found the accuracy attainable to be but little

better than from direct weighing. The contact finally adopted was a *long* piece of platinum wire, dipping into a *deep* container of mercury. The mercury must be kept scrupulously clean. When the steelyard is in balance, the point of balance being at the surface of the mercury, any oscillation will make
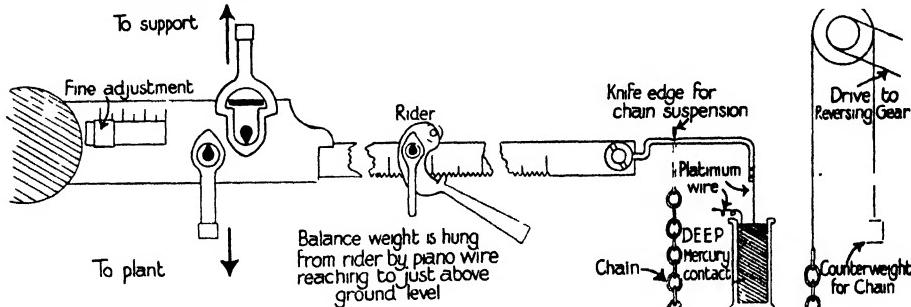
FIG. 2. Detail of steelyard, chain attachment, and contacts.

and break contact for approximately equal periods, the time of contact being independent of the amplitude of the oscillation. Consequently, when the rate of feed of chain to and from the steelyard is low, i.e. when the amount fed during one half-oscillation is not enough appreciably to affect the balance, it will be fed to, and removed from, the steelyard for the same periods, and balance will be maintained. This is easily arranged, and in practice the rate of chain-feed is adjusted to be only just enough to compensate for the maximum expected rate of weight-change. Under these conditions the sensitivity claimed by Balls was considerably exceeded. Any restriction of the amplitude of oscillation (which rarely exceeds 4° and is generally 1°–2°) has, in my experience, resulted in a decrease of sensitivity.

3. *The recording drum.* The pen operating on this drum is attached to the reversing gear in such a manner that its movements reflect those of the chain. Consequently it records the changes in weight of the load applied to the steelyard. Since the actual result desired is the rate of change of weight, a steadily revolving drum is not used, for the tangent to the curve at any time is not easily measured from a continuous curve. For this reason the drum illustrated in Fig. 3 was designed, and has proved very successful.

The drum itself is 6 in. in diameter, and 3 ft. long. It rotates on roller bearings, and one axle carries a rod 10 in. long radially to the drum. To this rod is attached a small spring-loaded pawl, engaging with a rubber band stretched round the drum itself. The weight of the rod and pawl is just sufficient to rotate the drum slowly, but the travel of the rod is so limited by stops that a full up-and-down movement rotates a spot on the surface of the drum about ⅛ in. An electromagnet is arranged as shown to lift the rod on the completion of a circuit; the breaking of this circuit releases the rod, which then falls gently against its stop, rotating the drum the predetermined amount.

The pen is attached to a brass plate moving on a geometric slide attached to the base-board. The drive to this plate is by an endless cord, wrapped once round an appropriately cut worm on a horizontal rod. Thus any rotation of this rod causes an appropriate travel of the pen.

During the operation of the automatic machinery the pen traces a horizontal line on the chart. At intervals (generally of five minutes) a momentary
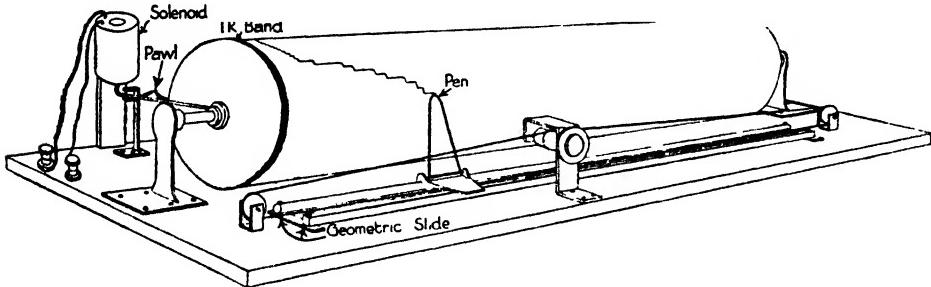
FIG. 3. Recording drum.

current is passed through the electromagnet, interrupting the line. The weight-loss is therefore traced out as a stepped curve, the lengths of the horizontal portions being directly related to the rate of weight-change. Thus a single measurement by a scale and multiplication by an appropriate factor give the rate of weight-loss without further complications.

METEOROLOGICAL DATA

Temperature and humidity. These data were obtained from a Casella thermo-hygrograph installed in a Stevenson screen. They were frequently checked but were never found to be seriously in error.

Saturation deficit. This was calculated from the thermo-hygrograph records.

I realize that such determinations are far from exact, as has, indeed, been pointed out by Kirkpatrick (1935). No other method was practicable, however, under the conditions of the work. This method of measurement, moreover, does not permit of the recording of any rapid variations.

Radiation. The vertical component of total solar radiation was measured by a Gorczinzky pyrheliometer, this instrument comprising a horizontal Moll thermopile under an evacuated glass dome. The E.M.F. produced is directly proportional to radiation, and was measured by an African assistant reading a galvanometer at sixty-second intervals. Latterly a Cambridge dot recorder was used.

Wind. The only anemometer available was a Fuess revolving-cup instrument. This is reasonably sensitive, but suffers from the unavoidable defect of all mechanically operated anemometers in that it possesses inertia. Thus a gust of wind towards the end of a period will inevitably affect the reading obtained during the next period. The anemometer was installed near the experimental tree, and read every sixty seconds throughout the day.

Wet-bulb depression, which could easily be continuously measured, served as a check on the determinations of the saturation deficit to which it closely approximates.

The following instrument was used for this purpose.

A copper-constantin thermopile, as illustrated in Fig. 4, with all its junc-
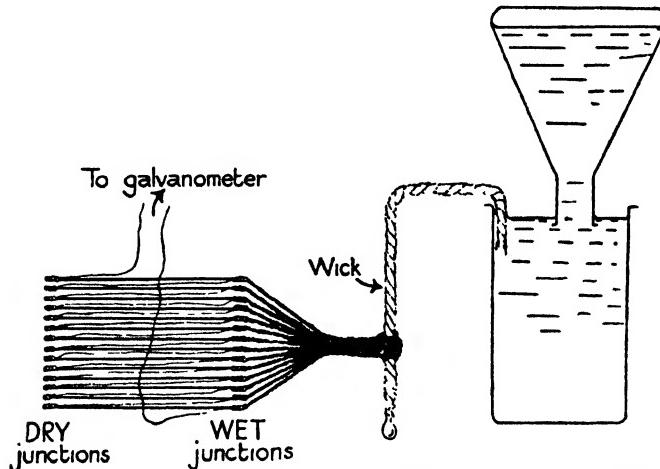
FIG. 4. Thermopile method for continuous recording of wet-bulb depression.

tions lagged with linen thread, and one set connected with similar thread to a moist wick, is placed in a wind tunnel, where it gives an E.M.F. directly proportional to the wet-bulb depression. The lagging of both junctions is necessary, otherwise minor and transitory variations in air temperature impart a fictitious irregularity to the readings. Records were taken every sixty seconds by means of a galvanometer, and latterly by a Cambridge dot recorder.

RESULTS AND DISCUSSION

Since the results of this work fall under separate heads, and since discussion under each head is called for, I have preferred to bring each set of results into juxtaposition with its discussion, rather than follow the customary procedure. Considerations of space make it impossible to present the complete data, for these include over 4,300 separate determinations of transpiration rate, nearly 35,000 of solar radiation, 11,000 of wind, 7,000 of wet-bulb depression, and continuous records of temperature and humidity over several months. Where selections from the bulk data have had to be made I have taken care that they should be representative.

The Detailed Daily March of Transpiration Rate

The short-period determinations of transpiration rate plotted in order give a reasonably accurate picture of the true march of transpiration rate throughout the day. Figs. 5 and 6 illustrate typical results, the transpiration
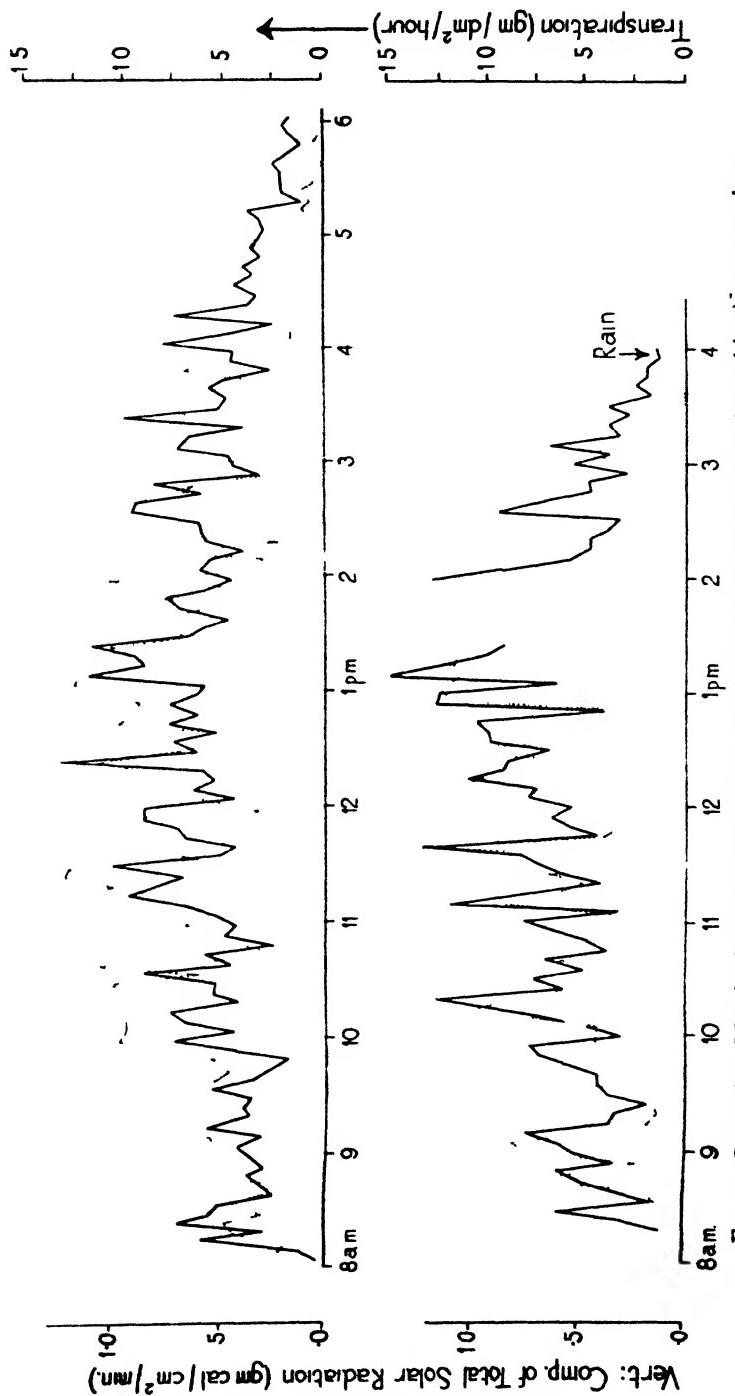
FIG. 5. *C. arabica*. March of transpiration rates (entire line) and of solar radiation (dotted line) over two days.

rates being recorded over 5-minute periods, and the radiation values being the average over the same periods. With the exception of part of Fig. 6, where wind velocity is indicated by vertical lines, no other meteorological factors are included. Fig. 7 illustrates a typical day's results under a hessian shade. The data for solar radiation were obtained outside the shaded area.
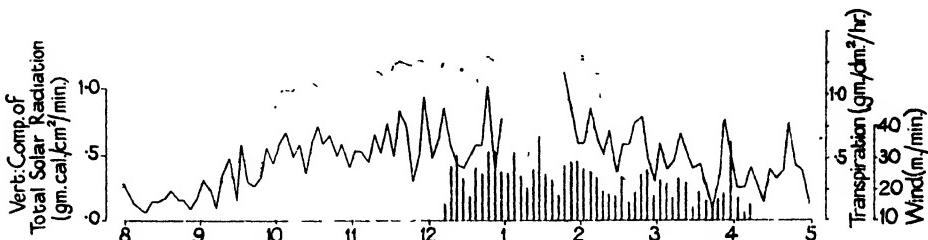
FIG. 6. *C. arabica.* March of the transpiration rate (entire line), of solar radiation (dotted line), and of wind velocity (vertical lines).

The most marked characteristic of these results is the large fluctuations in transpiration rate. It is of primary importance to decide whether these fluctuations correspond to actual changes in weight of the tree and its container, or whether they are illusory, due to either the operation of some external factor producing a variable force acting on the balance or to some imperfection in the machinery itself. Both of these possibilities are negatived by the following considerations.

1. Any phenomenon involving the expansion of the metal of the steelyard cannot account for the variations, since they are absent when any invariable weight is placed on the balance and are not reduced by enclosing the steelyard in a wooden box.

2. Strong winds affect the sensitivity of the balance, but work was always confined to days on which wind velocity rarely exceeded 40 m. per minute. Under similar conditions at night, when weight-losses of about 0·25 gm./min. are usual, or during exceptionally dull weather when they are usually about 5 gm./min., these fluctuations are absent although the balance immediately responds to small weights applied to it. This evidence, I think, is almost conclusive.

3. If all the leaves are removed from a tree, and replaced by pieces of paper similar in size, number, and disposition, fluctuations are absent, even in slightly gusty weather.

4. An average fluctuation corresponds to a weight-change of about 80 gm., spread over five minutes. The intermittent placing and removal of a weight of one-quarter of this amount on the balance results in an immediate and readily perceptible alteration in the *tempo* of the operation of the gear. This sudden change in *tempo* does not occur during the normal operation of the apparatus. Wind, if it was causing the fluctuations, must therefore operate gradually, both in increasing the weight and in decreasing it. Observation of air movements does not support this hypothesis.

5. It is just possible that the balance might be too sluggish to follow weight-changes accurately, and that a steady loss of weight might be thus converted into an apparently irregular one. Also, as previously stated, the reversing gear is set to feed chain from the balance at such a rate as only just to compensate the fastest expected rate of weight-change.

An increase in the rate of chain movement, although permitting of greater errors, should thus permit of any rapid, parasitic fluctuations to be recorded, and, if they are induced by wind or by some other external factor, occasional negative records should be obtained. Similarly, a shortening of the time-interval between rotatory movements of the recording drum should result in similar occasional negative records. Neither of these results is encountered in practice, and no change in the character of the records is apparent when the rate of chain movement is increased by fifteen times (enabling a weight-change of 80 gm. to be compensated in fifty-four seconds) or when the time-interval is shortened to thirty seconds. Consequently I believe that the changes in weight recorded by the balance are real, and correspond to actual changes in the transpiration rate of the trees under investigation.

It is also apparent that there is a general relationship between the fluctuations of transpiration rate and of radiation, more especially in the morning and evening. During the midday hours the correspondence is not so well marked and, as in Fig. 6, may be imperceptible.

It can be stated that the transpiration rate at any particular moment is mainly, if not entirely, controlled by the following factors: (1) the incident radiation; (2) the saturation deficit of the air; (3) air movement; (4) stomatal aperture; (5) other 'internal' factors, such as water tension in the plant (Haines 1935), changes in protoplasmic permeability (Du Sablon 1913, Henderson 1926), and so on.

During the course of this work the continuous records of temperature and humidity showed no changes over short time-intervals such as might cause appreciable fluctuations in the saturation deficit. This evidence, in itself rather unconvincing (for the recording instruments were enclosed in a Stevenson screen which damps any climatic oscillations very considerably (Kirkpatrick 1935)), is substantiated by continuous records of wet-bulb depression with the apparatus already described. Consequently the fluctuating transpiration rate cannot be ascribed to fluctuations in the evaporating power of the air, and this factor can be eliminated from the discussion.

Of the internal factors, water tension in the plant cannot reasonably be expected to exhibit rapid fluctuation, and in any event would exert little or no influence during the early hours of the day, when its value is demonstrably low but when fluctuations of transpiration rate are apparent.

Evidence has already been presented (Nutman 1937) that the stomata of *C. arabica* are light-sensitive and tend to open in radiation of moderate intensity, and to close when values of about o·9 gm. cal./cm.²/min. are exceeded: and stomatal movement is very rapid indeed at the temperatures of

a coffee plantation. This work, which was confined to the phenomena associated with horizontal leaves, does not, of course, enable the average stomatal aperture of a coffee tree to be predicted with any approach to accuracy: much must depend on the proportion of leaves shaded by others, and on the varying angles at which the incident radiation falls on them.

It is legitimate to suppose, however, that the stomatal behaviour of a whole coffee tree, taking into consideration its habit, is not grossly dissimilar from that of any single leaf. I should expect a direct relationship to hold between radiation and stomatal aperture up to a critical value (this being considerably higher than for a single leaf) merging into an inverse relationship at higher values of radiation. The stomata of a single leaf respond rapidly and completely to changes in radiation, and the average stomatal aperture of a tree would probably respond just as rapidly, but much less completely.

On any cloudless day the march of air-temperature is remarkably steady. This is not true for a day with intermittent clouds as Kirkpatrick has shown. Air temperature, however, has remarkably little effect on transpiration rate, as will appear later, and so cannot cause the fluctuations under discussion. Under any normal conditions, therefore, a reasonably steady march of saturation deficit and of water tension in the plant is contrasted with fluctuating wind velocity, stomatal aperture, and radiation. The varying transpiration rate is probably related to the variations in the three latter factors. Indeed, a glance at Figs. 5 and 6 is enough to show that radiation and transpiration vary together during much of the day.

Radiation certainly affects transpiration, partly by supplying energy for the evaporation of water, and partly through its effect on the permeability of protoplasm (Ivanov and Thielmann 1923; Henderson 1926). Also, as has already been shown, it affects the stomata. Consequently transpiration should be highly correlated with radiation at moderate radiation intensities, but less correlated at higher values. The following table illustrates the correlation coefficients between solar radiation and transpiration over five-minute periods, both in the open and inside the plantation. The data available under shade are adequate for similar correlations to be worked out.

TABLE I

The Relationship, as illustrated by the Correlation Coefficient, between the Transpiration Rate of C. arabica *and the Vertical Component of Total Solar Radiation, both taken over Five-Minute Intervals, in the Open and in a Coffee Plantation*

Site		Time of day								
		a.m.						p.m.		
		8–9	9–10	10–11	11–12	12–1	1–2	2–3	3–4	4–5
In open .	.	0·69	0·65	0·41	0·04	0·09	*0·41*	0·60	*0·32*	*0·29*
Inside plantation .		0·84	0·51	0·41	0·43	0·40	*0·21*	0·59	0·40	0·01

Coefficients significant to the 1 per cent. level are shown in heavy type: those to the 5 per cent. level in italics. This system of indicating the significance of correlation coefficients is used throughout this paper.

It will thus be seen that a high correlation in the morning diminishes during the midday hours and increases during the afternoon. That the correlation during the period 4–5 p.m. is not significant is explained by the fact that the sun was then very low, and long shadows fell on the experimental trees. No such shadows fell in the early morning period.

It should be noted that in the open, with an average midday radiation
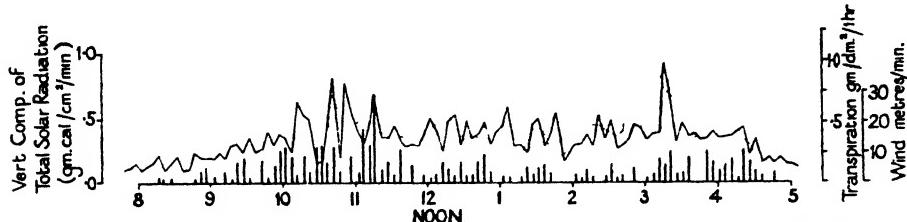
FIG. 7. *C. arabica*. March of transpiration (entire line) and of wind (vertical lines) under shade. The dotted line represents total solar radiation outside the shaded area.

intensity of about 1·2 gm. cal./cm.^{2}/min., the correlation coefficient is not significant at midday; in the plantation, with a radiation value of about 0·9 gm. cal./cm.^{2}/min., it is positive and just significant. Under hessian shade the data are insufficient for a similar study; but the correlation coefficient between transpiration rate (t) and solar radiation (r) between the hours of 11 a.m. and 1 p.m. is

$$^r\text{tr} = 0.32$$

The only other variable meteorological factor for which data over five-minute intervals are available is wind, and then for only five days in the plantation, and for the same time under shade. Inside the plantation the correlation between wind and transpiration rate fails to reach the level of significance at any time. In the shade, however, the correlation coefficients between the five-minute values of transpiration rate (t), total solar radiation (r), and wind velocity (w) are as follows:

$$^r\text{tw} \quad - 0.41 \qquad ^r\text{tr} \quad - 0.56$$
$$^r\text{tw.r} \quad - 0.28 \qquad ^r\text{tr.w} \quad 0.49$$

The data are hardly adequate for generalization, and further work on this subject is desirable. It certainly appears, though, as if wind had an enhanced effect when the stomata were open. That air movements would affect transpiration most when the wet cell-walls of the mesophyll are in direct communication with the outside air appears reasonable, although Seybold (1929, 1931, 1933) quoted by Miller (1938) states that wind affects cuticular transpiration more than that through the stomata. Seybold's original papers are not available to me.

The observed short-period fluctuations in the transpiration rate of *C. arabica*, therefore, are related both to incident radiation and to air movements, but not to air temperature, or to water strain in the plant, nor in all probability to saturation deficit. Radiation apparently acts in a twofold

TABLE II

March of Transpiration Rates of Coffea arabica and of Meteorological Factors (all expressed as Hourly Averages) in an Exposed Situation in March 1939

T = Transpiration rate (gm./dm.²/hr.)
R = Vertical component of total solar radiation (gm. cal./cm.²/min.)
θ = Air temperature (°C.)
S = Saturation deficit (millibars)

Hours	11/3 T	11/3 R	12/3 T	12/3 R	13/3 T	13/3 R	14/3 T	14/3 R	15/3 T	15/3 R	6/3 T	6/3 R	6/3 θ	6/3 S
8–9	0·88	0·50	—	—	0·61	0·35	0·78	0·44	1·00	0·53	0·79	0·47	24	16
9–10	—	—	0·72	0·64	0·90	0·72	0·97	0·76	1·16	0·87	1·11	0·85	26	21
10–11	1·11	1·23	1·19	0·90	1·10	0·79	1·19	0·99	1·49	1·05	1·36	1·14	28	27
11–12	1·29	1·26	1·17	1·17	1·21	1·17	1·37	1·23	0·82	1·14	1·43	1·31	29	28
12–1	1·14	0·99	1·06	1·20	1·17	1·02	1·31	1·17	0·82	1·23	1·46	1·37	30	32
1–2	0·88	0·50	1·32	0·88	0·91	0·41	0·94	0·96	0·65	0·88	1·61	1·17	30	33
2–3	—	—	—	—	0·78	0·44	1·01	0·79	0·79	0·94	1·35	0·82	29	31
3–4	—	—	—	—	0·73	0·29	0·77	0·32	0·93	0·70	1·10	0·50	29	31
4–5	—	—	—	—	0·59	0·11	0·87	0·29	0·89	0·20	0·97	0·50	28	29
Average	1·06	0·90	1·11	0·95	0·90	0·59	1·02	0·77	0·95	0·84	1·24	0·90	26	28

Hours	9/3 T	9/3 R	9/3 θ	9/3 S	10/3 T	10/3 R	10/3 θ	10/3 S	16/3 T	16/3 R	16/3 θ	16/3 S	Averages of days 6/3, 9/3, 10/3, 16/3 T	R	θ	S	Averages of all days T	R
8–9	0·53	0·26	19	6	—	—	—	—	0·73	0·38	26	17	0·76	0·33	22	13	0·76	0·42
9–10	1·02	0·55	21	7	0·54	0·44	20	6	0·91	0·61	26	17	0·89	0·61	23	13	0·93	0·68
10–11	1·35	0·85	23	10	0·94	0·76	22	9	1·20	0·93	27	21	1·21	0·92	25	17	1·23	0·93
11–12	1·52	0·93	25	13	1·23	0·94	23	19	1·13	1·17	29	26	1·32	1·09	27	22	1·22	1·14
12–1	1·64	1·20	27	17	1·46	1·31	26	21	1·20	1·11	29	26	1·44	1·27	28	24	1·27	1·21
1–2	1·64	0·93	27	20	1·63	1·26	27	25	1·01	1·14	31	29	1·48	1·12	29	27	1·20	0·96
2–3	1·27	0·70	28	22	1·41	0·90	28	29	0·90	0·73	31	30	1·23	0·79	29	28	1·05	0·73
3–4	1·13	0·70	28	23	1·13	0·70	29	30	0·74	0·50	29	28	1·02	0·60	29	28	0·93	0·53
4–5	0·90	0·23	28	23	0·85	0·50	29	32	0·60	0·44	29	27	0·83	0·42	29	28	0·71	0·32
Average	1·22	0·71	25	16	1·15	0·85	29	22	0·93	0·78	28	25						

manner, in part through its effect on the stomata, resulting when its intensity is high in a reduction of its normal accelerating effect.

Wind plays a secondary role: its effect is only significant under shade, possibly due to a larger stomatal aperture there.

The March of Hourly Average Transpiration Rates

Tables II and III and Fig. 8 summarize the available information for the two main stations, all data appearing therein as hourly averages. Complete data for the open are only available for the dates 6/3/39, 9/3/39, 10/3/39, and 16/3/39, although transpiration and radiation are included for 12/3/39 to 15/3/39 inclusive. Wind measurements are only available for the plantation, and then for only five days. Table IV summarizes the results obtained under shade. Radiation is measured under the shade, except on the first and the last day of the work, when it was measured outside in order to give some idea of the prevailing external conditions. The fact that only one solarimeter was available precluded simultaneous observations both inside and outside the shaded area. Fig. 9 illustrates the results.

These data, of course, are directly comparable with those of other workers using methods of direct weighing.

It should first be made clear that the markedly different levels of environmental conditions in the three graphs should not be referred to the differences in situation. Kirkpatrick (1935) found in a coffee plantation that 'by day it is generally warmer and drier, though with a cloudy sky it may be warmer and damper. Only when the bushes are wet is it cooler and damper during the middle of the day, and as is perhaps to be expected, there is no occasion when the plantation is cooler and drier than the screen.' The screen referred to was installed in the open.

Kirkpatrick's results, supported as they are by a considerable and adequate weight of evidence, show definitely that the general difference between conditions outside and inside a coffee plantation is other than would appear from a cursory reference to Fig. 7. The explanation of this apparent discrepancy is plain from the data for solar radiation in the two situations, since it was much more sunny when records were taken in the open than when work was proceeding in the plantation, other environmental factors being also more extreme as a consequence. Had the work at the two stations been conducted simultaneously it is reasonably certain that the conditions in the plantation would have been the more extreme. Because of this the transpiration data should be referred to the climatic environments as defined in the tables, and not to the respective positions of the plants.

For a variety of reasons the volume of data for each set of conditions varies, and as a consequence treatments that can be applied to one set of results are inapplicable to others. I have preferred to treat each set of data as fully as possible, rather than to limit the treatment to that imposed by the least adequate. Consequently the following tables are not all similar.
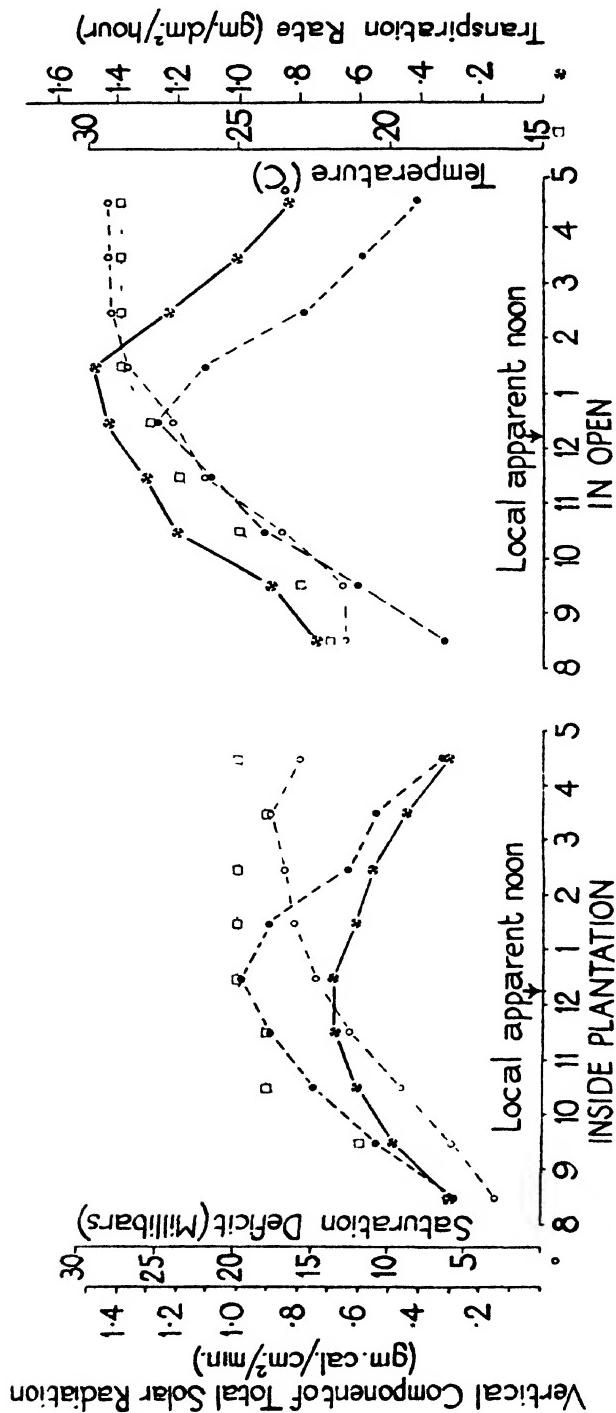
FIG. 8. *C. arabica.* March of transpiration rates and of meteorological factors (all expressed as hourly averages) for two different environments. Although both sets of data were obtained during the same season of the year, they represent successive periods.

TABLE III

March of Transpiration Rates of C. arabica and of Meteorological Factors (all expressed as Hourly Averages) inside an Unshaded Plantation in March and April 1939

T = Transpiration rate (gm /dm.² hr.)
R = Vertical component of total solar radiation (gm cal cm⁻² min)
θ = Temperature (°C.)
S = Saturation deficit (millibars)
W = Wind velocity (m /hr.)

Hours	21/3 T	R	θ	S	25/3 T	R	θ	S	26/3 T	R	θ	S	27/3 T	R	θ	S	28/3 T	R	θ	S	29/3 T	R	θ	S	31/3 T	R	θ	S	W	Average T	R	θ	S
8–9	—	—	—	—	0.73	0.50	19	4	0.60	0.54	19	4	0.48	0.64	22	8	0.12	0.19	18	7	0.42	0.55	20	6	0.50	0.56	21	7	2260	0.30	0.29	18	3.0
9–10	0.27	0.29	22	4.0	0.73	0.67	22	8	0.55	0.59	21	8	0.50	0.84	24	11	0.68	0.61	19	11	0.72	0.92	23	12	0.54	0.62	21	8	2405	0.48	0.53	21	5.8
10–11	0.65	1.03	26	11.0	0.57	1.00	23	12	0.46	0.81	21	8	0.55	1.05	26	15	0.81	0.71	23	14	0.96	1.07	25	15	0.68	0.88	23	10	2110	0.61	0.75	24	9.1
11–12	0.65	1.13	23	12.0	0.58	0.97	26	13	0.55	0.73	24	13	0.51	0.88	27	16	0.90	0.83	24	17	0.80	0.89	26	19	0.65	0.81	24	12	2250	0.68	0.89	24	12.5
12–1	0.50	0.84	23	15.0	0.54	0.67	25	14	0.55	0.70	21	12	0.53	0.81	27	17	0.92	0.90	26	20	0.73	0.90	27	20	0.64	0.89	25	15	2145	0.69	0.99	25	14.6
1–2	0.33	0.51	22	10.0	—	—	—	—	—	0.84	19	10	0.26	0.37	25	17	0.56	0.71	27	20	0.53	0.63	27	22	0.48	0.63	25	15	2683	0.62	0.65	25	15.9
2–3	—	—	—	—	—	—	—	—	0.29	0.75	21	11	0.27	0.19	26	13	0.35	0.59	27	20	0.35	0.31	24	16	0.44	0.75	24	14	—	0.56	0.54	25	16.6
3–4	—	—	—	—	0.17	0.57	27	20	0.22	0.19	21	11	0.18	0.39	25	15	—	0.25	26	22	—	—	—	—	—	—	—	—	—	0.45	0.33	24	17.5
4–5	—	—	—	—	0.22	0.34	27	19	—	0.38	20	11	—	—	—	—	0.35	—	26	—	—	—	—	—	—	—	—	—	—	0.32	—	25	15.7
Average	0.48	0.76	23	10.4	0.53	0.74	24	13	0.48	0.64	21	10	0.41	0.65	25	14	0.61	0.66	24	15	0.68	0.80	25	17	0.56	0.73	23	12	—	—	—	—	—

Hours	2/4 T	R	θ	S	W	3/4 T	R	θ	S	W	4/4 T	R	θ	S	W	21.4 T	R	θ	S	W	25.4 T	R	θ	S	W
8–9	0.12	0.10	17	2	60	0.38	0.37	17	2	1080	—	—	16	—	1490	0.01	0.15	18	4	95	0.46	0.48	20	4	120
9–10	0.60	0.46	18	3	245	0.42	0.62	20	6	1075	0.14	0.44	22	8	2280	0.43	0.28	20	5	200	0.68	0.62	23	8	335
10–11	0.56	0.58	21	10	960	0.54	0.91	21	14	1505	0.48	0.82	23	12	2075	0.60	0.40	22	8	410	0.85	0.80	24	10	415
11–12	0.72	0.91	23	14	1520	0.75	0.97	23	15	1860	0.64	0.83	24	15	1415	0.35	0.30	22	9	430	1.11	0.93	25	13	300
12–1	0.87	1.11	25	15	1910	0.69	0.78	26	18	1355	0.83	0.86	24	16	1860	0.42	0.42	22	9	210	0.96	0.84	26	15	845
1–2	0.85	0.99	26	15	1390	0.77	0.85	26	18	1095	0.80	0.72	24	17	1760	0.28	0.41	22	9	95	0.73	0.46	24	11	320
2–3	0.66	0.69	26	16	760	0.63	0.62	26	19	1510	0.63	0.53	25	17	2135	0.57	0.44	23	9	100	0.67	0.71	26	15	705
3–4	0.47	0.31	26	16	1170	0.54	0.62	26	18	1845	0.59	0.52	24	18	—	0.28	0.27	23	10	265	0.36	0.33	24	12	410
4–5	0.41	0.19	25	17	—	0.45	0.32	25	18	2260	0.45	0.35	—	—	—	—	—	—	—	190	—	—	—	—	—
Average	0.58	0.59	23	11	—	0.57	0.68	23	13	—	0.61	0.64	23	13	—	0.37	0.35	22	8	—	0.73	0.64	25	11	—

March of Transpiration Rates of C. arabica and of Meteorological Factors (all expressed as Hourly Averages) inside an Unshaded Plantation in April 1939. The Experimental Tree was shaded by Hessian Cloth

Excepting for total solar radiation and wind, the meteorological data were measured outside the shaded area. The temperature and humidity inside did not differ appreciably from that outside. On 5/4 and 10/4 solar radiation was measured outside the shade.

Notation as in Table III

5/4

Hours	T	R	θ	S	W
8–9	0.16	0.38	18	4.0	0
9–10	0.34	0.50	19	6.5	0
10–11	0.57	1.13	22	10.5	0
11–12	0.61	1.17	23	14.0	0
12–1	0.61	1.27	23	15.0	1500
1–2	0.75	1.11	24	15.0	1885
2–3	0.59	0.68	23	15.0	1380
3–4	0.50	0.47	23	14.5	1180
4–5	0.32	0.26	23	15.0	470
Average	0.49	0.77	22	12.2	713

10/4

Hours	T	R	θ	S	W
8–9	0.15	0.35	18	3.0	70
9–10	0.20	0.48	19	5.0	40
10–11	0.51	0.91	21	7.5	410
11–12	0.57	1.12	23	11.0	435
12–1	0.61	0.95	24	13.0	455
1–2	0.65	0.99	25	15.0	485
2–3	0.59	0.40	25	15.5	165
3–4	0.38	0.38	25	15.5	170
4–5	0.33	0.47	24	14.0	195
Average	0.44	0.67	23	11.1	731

6/4

Hours	T	R	θ	S	W
8–9	0.21	0.035	17	2.0	10
9–10	0.36	0.073	18	4.0	695
10–11	0.43	0.082	22	10.0	985
11–12	0.53	0.109	24	12.5	840
12–1	0.46	0.082	24	14.0	780
1–2	0.53	0.073	26	16.0	1045
2–3	0.48	0.113	25	15.5	600
3–4	0.49	—	24	15.0	—
4–5	0.29	—	24	15.0	—
Average	0.42		23	11.6	

7/4

Hours	T	R	θ	S	W
8–9	0.12	0.045	18	2.5	15
9–10	0.28	0.149	19	4.0	155
10–11	0.41	0.152	22	8.5	355
11–12	0.53	0.181	24	13.0	410
12–1	0.61	0.272	24	14.0	755
1–2	0.69	0.285	26	16.0	1420
2–3	0.59	0.194	26	18.0	630
3–4	0.49	0.130	27	19.0	305
4–5	0.28	0.079	26	18.0	580
Average	0.44	0.165	24	12.6	514

8/4

Hours	T	R	θ	S	W
8–9	0.15	0.043	19	4.5	50
9–10	0.41	0.153	22	8.0	475
10–11	0.44	0.177	23	10.5	20
11–12	0.67	0.176	26	15.0	955
12–1	0.67	0.255	27	18.0	1035
1–2	0.70	0.214	27	19.0	1390
2–3	0.61	0.185	27	17.0	1885
3–4	0.35	0.075	24	14.0	880
4–5	0.17	0.022	23	11.0	35
Average	0.46	0.144	24	13.0	747

Average of 6/4, 7/4, 8/4

Hours	T	R	θ	S	W
8–9	0.16	0.041	18.0	3.5	25
9–10	0.35	0.125	19.7	5.3	441
10–11	0.42	0.137	22.3	9.6	440
11–12	0.57	0.155	24.7	13.5	735
12–1	0.65	0.203	25.0	15.3	860
1–2	0.64	0.191	26.3	17.0	1285
2–3	0.56	0.167	26.0	16.8	1038
3–4	0.44	0.068	25.0	16.0	557
4–5	0.25	0.030	24.3	14.7	307

Average of 5/4, 7/4, 8/4, 10/4

	Average of 5/4 and 10/4				
	R	T	θ	S	W
8–9	0.36	0.14	17.9	4.0	29
9–10	0.49	0.32	19.6	5.4	273
10–11	1.02	0.47	22.1	9.5	354
11–12	1.14	0.58	23.9	13.1	882
12–1	1.11	0.59	24.6	14.8	905
1–2	1.00	0.66	25.4	16.2	1239
2–3	0.54	0.57	25.2	16.2	972
3–4	0.42	0.44	24.7	14.1	634
4–5	0.36	0.28	24.2	14.5	320

FIG. 9. *C. arabica.* March of transpiration rates and of meteorological factors (all expressed as hourly averages) inside an unshaded plantation, the experimental tree being shaded by hessian cloth.

TABLE V

The Relationships, as illustrated by the Correlation Coefficients, between the Hourly Average Values of the Transpiration Rate (t) of C. arabica, *Total Solar Radiation (r), Air Temperature (θ), and Saturation Deficit (s), in an Open Situation*

r_{tr}	**0·77**	r_{rs}	0·27	$r_{s\theta}$	**0 93**
r_{ts}	0·28	$r_{r\theta}$	0·31		
$r_{t\theta}$	0·32				
$r_{tr.\theta}$	**0·74**	$r_{ts.\theta}$	−·06	$r_{t\theta.s}$	0·17
$r_{tr.s}$	**0·73**	$r_{ts.r}$	0·11	$r_{t\theta.r}$	0·13
$r_{tr.\theta s}$	**0·72**	$r_{tr.\theta r}$	−·03	$r_{t\theta.sr}$	0·08

TABLE VI

The Relationships, as expressed by the Correlation Coefficients, between the Hourly Average Values of the Transpiration Rate (t) of C. arabica, *Solar Radiation (r), Saturation Deficit (s), and Air Temperature (θ) inside an Unshaded Plantation*

r_{tr}	**0·77**	r_{rs}	**0·30**	$r_{s\theta}$	**0·89**
r_{ts}	**0·34**	$r_{r\theta}$	**0·35**		
$r_{t\theta}$	**0·41**				
$r_{tr.\theta}$	**0·74**	$r_{ts.\theta}$ = −·05	$r_{t\theta.s}$ − *0·26*
$r_{tr.s}$ − **0·75**	$r_{ts.r}$	0·18	$r_{t\theta.r}$ = *0·23*
$r_{tr.s\theta}$ − **0·74**	$r_{ts.r\theta}$ − −·06	$r_{t\theta.rs}$ = 0·15

TABLE VII

The Relationships, as expressed by the Correlation Coefficients, between the Hourly Average Values of the Transpiration Rate (t) of C. arabica, *the Solar Radiation (r), and the Saturation Deficit (s) inside an Unshaded Plantation*

Time of day	r_{tr}	r_{ts}	r_{rs}	$r_{tr.s}$	$r_{ts.r}$
8–10 a.m.	0·77	0·54	0·68	0·65	0·04
10 a.m. to 12	0·51	0·48	0·68	0·28	0·21
12–2 p.m.	0·62	0·59	0·51	0·46	0·36
2–4 p.m.	0·70	0·49	0·28	0·67	0·42

TABLE VIII

The Relationships, as expressed by the Correlation Coefficients, between the Hourly Average Values of the Transpiration Rate (t) of C. arabica, *the Air Temperature (θ), the Saturation Deficit (s), and the Wind Velocity (w) inside a Plantation, the Experimental Material being shaded with Hessian Cloth*

$r_{tr} = 0·79$	$r_{rs} = 0·65$	$r_{s\theta} = 0·98$	$r_{\theta w} = 0·72$
$r_{ts} = 0·86$	$r_{r\theta} = 0·61$	$r_{sw} = 0·72$	
$r_{t\theta} = 0·86$	$r_{rw} = 0·51$		
$r_{tw} = 0·77$			
$r_{tr.s} = 0·59$	$r_{ts.r} = 0·75$	$r_{tw.r} = 0·71$	$r_{t\theta.r} = 0·77$
$r_{tr.\theta} = 0·65$	$r_{ts.\theta} = 0·20$	$r_{tw.s} = 0·43$	$r_{t\theta.s} = 0·13$
$r_{tr.w} = 0·72$	$r_{ts.w} = 0·69$	$r_{tw.\theta} = 0·44$	$r_{t\theta.w} = 0·69$
$r_{tr.s\theta} = 0·63$	$r_{ts.\theta r} = 0·03$	$r_{tw.\theta s} = 0·43$	$r_{t\theta.rs} = 0·26$
$r_{tr.sw} = 0·62$	$r_{ts.\theta w} = 0·15$	$r_{tw.\theta r} = 0·48$	$r_{t\theta.rw} = 0·62$
$r_{tr.\theta w} = 0·66$	$r_{ts.wr} = 0·58$	$r_{tw.rs} = 0·48$	$r_{t\theta.ws} = 0·12$

$$r_{tr.sw\theta} = 0·65$$
$$r_{ts.rw\theta} = -·05$$
$$r_{t\theta.rws} = 0·28$$
$$r_{tw.rs\theta} = 0·48$$

The results show quite clearly that in all three situations radiation plays the preponderating part in determining the average hourly water loss. Saturation deficit plays no part in the open, nor (except in the late afternoon) inside the plantation, nor under shade. Wind significantly increases the hourly average rate of water loss under shade, but the absence of data makes it impossible to estimate its effect in the open or inside the unshaded plantation.

The surprisingly high value of transpiration rate under shade can only be adequately explained by stomatal behaviour. It will be seen that, apart from radiation, the meteorological factors were not appreciably different from those in the unshaded plantation, and consequently, if the shade were removed, a rate of about 0·5 gm./dm.^{2}/hr. might have been expected. The rate actually attained was 0·45 gm./dm.^{2}/hr. Thus, although the radiation was reduced by 70 per cent., the concomitant reduction in water loss was only 10 per cent.

These correlations should be contrasted with those published by Briggs and

Schantz (1916), who worked on the hourly average transpiration of a number of plants. They show that, under the conditions of their experiments, 'the correlation coefficients of transpiration with radiation range from 0·82 to 0·89; with temperature from 0·77 to 0·86; with wet-bulb depression from 0·75 to 0·85'.

It is worth pointing out that their coefficients have been derived from data extending over the whole twenty-four hours of the day. While it seems at first sight logical to include all data, yet a moment's consideration will show that as the actual object of the studies is the interaction of meteorological factors with transpiration, and since transpiration is negligible at night, and radiation is then absent and the associated factors reach a low level, any correlation coefficient must perforce be positive and highly significant, irrespective of the actual relationship between that factor and transpiration during the daytime. Consequently the interaction of factors controlling transpiration is best studied during that period when water losses are appreciable.

I therefore believe that the correlations published by these authors are of limited use in forwarding our understanding of transpiration. In order to test this belief, one of Briggs and Schantz's sets of results (that for sorghum) was chosen at random and the data for the period 6 a.m. to 6 p.m. analysed.

TABLE IX

Data from Briggs and Schantz

The Relationship, as expressed by the Correlation Coefficients, between the Hourly Average Values for Transpiration (t), Solar Radiation (r), Air Temperature (θ), Wet-bulb Depression (b), and Wind Velocity (w) for Sorghum.
(N.B. *The values for solar radiation are* not *the vertical component, but are measured normally to the incident radiation*)

r_{tr} 0·63
$r_{t\theta}$ 0·46 $r_{r\theta}$ − 0·41
r_{tb} 0·14 r_{rb} −·05 $r_{\theta b}$ − 0·78
r_{tw} 0·00 r_{rw} 0·51 $r_{\theta w}$ = −·34 r_{bw} = −·67

$r_{tr.\theta}$ 0·54 $r_{t\theta.r}$ − 0·28 $r_{tb.\theta}$ = −·39 $r_{tw.r}$ = −·48
$r_{tr.b}$ 0·65 $r_{t\theta.b}$ − 0·56 $r_{tb.r}$ 0·22 $r_{tw.\theta}$ = 0·19
$r_{tr.w}$ − 0·73 $r_{t\theta.w}$ − 0·49 $r_{tb.w}$ = 0·19 $r_{tw.b}$ = 0·22

$r_{tr.\theta b}$ − 0·42 $r_{t\theta.rw}$ = −·08 $r_{tb.\theta r}$ − −·07 $r_{tw.\theta r}$ = 0·41
$r_{tr.\theta w}$ − 0·63 $r_{t\theta.rb}$ =− 0·17 $r_{tb.\theta w}$ · 0·38 $r_{tw.\theta b}$ = −·12
$r_{tr.bw}$ 0·73 $r_{t\theta.bw}$ − 0·57 $r_{tb.rw}$ = 0·24 $r_{tw.rb}$ = −·52

$r_{tr.\theta bw}$ = 0·57
$r_{t\theta.brw}$ = 0·14
$r_{tw.\theta rb}$ = −·50
$r_{tb.\theta wr}$ = 0·48

All these final correlations, with the exception of $r_{t\theta.brw}$, are significant.

These results show clearly that the original primary correlations, indicating a positive relationship between transpiration and both radiation and temperature, are misleading. The interrelationships between the meteorological factors, seen in the primary correlations in the first section of the table, are such that transpiration is shown, in the final analysis, to be positively correlated with radiation and with wet-bulb depression, and negatively correlated with wind.

I do not wish particularly to stress the results derived from these Sorghum data, since they only cover three days and the unusual primary correlations unduly complicate the final analysis. But I desire to emphasize the complete dissimilarity of the final results from those given by the authors, derived from data extending over twenty-four hours.

The disappearance of temperature as a controlling factor of transpiration is, I think, to be expected. Temperature, in itself, can have but a limited effect, and its elimination on analysis is obviously due to its strong interrelations with both radiation and wet-bulb depression.

The Daily Transpiration Rate

The different levels of transpiration in the three different environments studied call for explanation. From a study of Figs. 8 and 9 it is apparent that radiation is not by any means the sole factor determining the total water loss, for the rates under shade are almost as high as those in an unshaded plantation, although the radiation is very much less. Also, the radiation in the open does not differ greatly from that inside the plantation, but the transpiration is very different indeed.

The following table summarizes the interrelations of transpiration and of the varying climatic factors. Wind data cannot be included, for they are absent for the open. Because of the fact that there are only eighteen days for which complete records for all factors are available, the results are not as conclusive as might be desired, and further work is necessary before the conclusions tentatively advanced can be accepted.

TABLE X

The Relationships, as expressed by the Correlation Coefficients, between the Average Daily Values (over Nine Hours) of the Transpiration Rate (t), of C. arabica, the Solar Radiation (r), the Saturation Deficit (s), and the Air Temperature (θ)

$$r_{tr} = 0.60 \qquad r_{rs} = 0.49 \qquad r_{\theta s} \quad 0.85$$
$$r_{ts} = 0.72 \qquad r_{r\theta} \quad 0.39$$
$$r_{t\theta} = 0.79$$

$$r_{tr.s} \quad 0.38 \qquad r_{ts.\theta} = 0.50 \qquad r_{t\theta.s} \quad 0.15$$
$$r_{tr.\theta} \quad 0.50 \qquad r_{ts.r} \quad 0.71 \qquad r_{t\theta.r} \quad 0.67$$

$$r_{tr.\theta s} = 0.40$$
$$r_{ts.\theta r} = 0.39$$
$$r_{t\theta.rs} = 0.16$$

It should be noted that, although none of the final correlation coefficients reaches the 5 per cent. level of significance, yet both $r$ts.θr and $r$tr.θs are only just below that level. When the extremely small amount of data is considered, the inconclusive results are not surprising. Such as they are, however, they substantiate the conclusions drawn by inspection of Figs. 8 and 9, namely, that the daily transpiration is affected largely by saturation deficit and that radiation does not play the preponderating part that it does when shorter periods of time are concerned. The very small correlation with temperature should be noted.

These results, as already stated, are in sharp contradistinction to the conclusions of Briggs and Schantz (1916), who studied the daily transpiration rates of a large variety of plants and related their results to meteorological factors. These are stated to be 'integrated for each day', but it is not definitely stated that they were measured over the whole twenty-four hours, although presumably this was so. Since, as has already been pointed out, the sum total of transpiration is virtually unaffected by the levels of climatic factors during the night, it appears inadvisable to include this period in the analysis.

Briggs and Schantz concluded that the daily transpiration was highly correlated with all factors measured excepting wind, this showing only a very small correlation. As in the case of sorghum I have chosen at random their data for wheat in the month of July, and analysed them in the following table.

TABLE XI

Data from Briggs and Schantz (1916)

The Interrelationships, as indicated by the Correlation Coefficients, between the Average Daily Transpiration (t) of Wheat, the Solar Radiation (r), the Wet-bulb Depression (b), and the Wind Velocity (w)

$r$tr	- 0·74	$r$rb	0·60	rbθ	0·78	rwθ	−·08
$r$tb	0·90	rrθ	0·54	$r$bw	0·36		
rtθ	0·78	$r$rw	0·31				
$r$tw	0·29						
$r$tr.b	0·59	$r$tb.r	0·86	rtθ.r	0·68	$r$tw.r	0·08
$r$tr.θ	0·62	$r$tb.θ	0·75	rtθ.b	0·29	$r$tw.b	−·11
$r$tr.w	0·72	$r$tb.w	0·90	rtθ.w	0·84	$r$tw.θ	0·56
$r$tr.θw	0·51	$r$tb.θw	0·61	$r$tw.θr	0·42	rtθ.rb	0·27
$r$tr.θb	0·58	$r$tb.θr	0·73	$r$tw.br	−·23	rtθ.wr	- 0·74
$r$tr.wb	0·62	$r$tb.wr	0·86	$r$tw.θb	0·10	rtθ.wb	0·29
$r$tr.θbw	0·58	$r$tb.rθw	0·67	rtθ.bwr	0·16	$r$tw.brθ	0·09

The primary correlations of this table are, naturally, in full agreement with the deductions of the authors, namely, that daily transpiration is controlled by radiation, wet-bulb depression, transpiration, and air temperature. Further analysis, however, shows that (for the part of their data studied)

transpiration is independent of wind and temperature and mainly controlled by radiation and wet-bulb depression. This result is in complete agreement with my tentative conclusions, although the plants studied, the method of study, and the conditions differ widely.

The Effect of Water Strain in the Plant

The small number of trees available necessarily reduced the amount of work of this type that could be done, since it is obviously undesirable to use for further records material that has shown even slight wilting. Table XII illustrates a typical record, this particular tree first showing signs of water strain at 11 a.m.

TABLE XII

The Transpiration Rates of C. arabica *suffering from just perceptible Wilting under the External Conditions defined in the Table*

Time	Trans. (gm./dm.²/hr.)	Radiation (gm. cal./cm.² /min.).	Temp. 'C.	Saturation deficit (millibars)
11 a.m.–12	0·55	1·03	18	15
12–1	0·45	1·01	27	21
1–2	0·40	1·03	28	28
2–3	0·36	1·03	29	29
3–4	0·27	0·61	31	31
4–5	0·22	0·37	32	32

It should be noted that these records were obtained on a day when solar radiation was well above the average value recorded inside the plantation, and other conditions were also exceptionally severe. All conditions therefore favoured very high transpiration, yet these rates are actually the lowest values recorded in the course of this work.

'Wilting' as referred to here is very slight indeed, and would pass unnoticed by anyone not familiar with the specific premonitory symptoms of water strain in *C. arabica*. At the same time it corresponds to demonstrably high water tension in the conducting tissues of the plant, and this, in my opinion, is undoubtedly the cause of the low rates of water loss recorded.

SUMMARY

1. A method is described whereby the transpiration rates of large plants can be determined over short time-intervals.

2. Results are presented for *Coffea arabica* in three different environments, together with the accompanying climatic factors.

3. Rapid and considerable short-period fluctuations in transpiration rates are described and illustrated, and evidence presented for their reality.

4. The details of the daily march of transpiration, recorded over five-minute intervals, are shown to be due almost entirely to variations in the incident radiation, especially when radiation is moderate or low. During periods of

high radiation the correlation is reduced, sometimes to insignificance. This is ascribed to the effects of stomatal movements.

5. The hourly average transpiration rate is also mainly determined by radiation, although other factors play a subsidiary part especially when radiation is moderate or low.

6. The daily transpiration rate is shown to be determined by both radiation and by saturation deficit, and to be independent of temperature.

7. These results are discussed in relation to those of Briggs and Schantz, and it is suggested that the deductions made by these authors from their records are in question.

8. The transpiration rate of coffee is shown to be very greatly reduced during periods of slight water strain, and this is ascribed to the high water tension in the plant at such times.

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The Breakdown of Meiosis in a Male-Sterile Saccharum

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With twenty-one Figures in the Text

INTRODUCTION

IN many hermaphrodite plants individuals occur with defective anthers. This defect arises in many ways, but it is always genetically determined (cf. Lewis, 1940), and may sometimes play an important part in the evolution of the genetic system (Frankel, 1940). Studying the precise means of breakdown, however, is of importance from an entirely different point of view. Beadle (1933) has shown that in maize there are some dozen genetically distinct determinants of male sterility, and these determinants act in many different ways. Some of them, such as the polymitotic gene, are already of great physiological interest and others await more exact interpretation.

Types of male sterility may be conveniently subdivided into those which act through the intermediacy of the chromosome-development and those which are seen directly to affect the spindle. Of the first the asynaptic gene in *Crepis capillaris* (Richardson, 1935) is an example; of the second, the breakdown of tetrad formation in Kniphofia (Moffett, 1932). Physiologically, a more obvious means of classification is that depending on the onset of breakdown. Abnormalities which in Chrysanthemum affect premeiotic divisions (Shimotomai, 1931) are at the one end of the scale; at the other extreme might be placed those types of pollen in hybrid species of Oenothera which although externally normal fail to germinate.

The types of male sterility which affect the development of the spindle are of particular interest for comparison with the results of experimental treatment and of non-pairing of chromosomes, which has always of course a secondary effect on spindle development. In a recent account Darlington and Thomas (1937) have been able to show in a Festuca-Lolium derivative that a failure in the combination of the separate spindles developed by the centromeres of each chromosome leads to a splitting of the spindle at anaphase and the formation at telophase of three nuclei instead of two. They also found that at the second division spindles could be developed which ran round the apparently quiescent nucleus and were developed, therefore, without any

relationship to the centromeres, from which the first division spindles were most palpably derived.

In the present study I am concerned with another grass, a derivative of *Saccharum officinarum* with 110 chromosomes. The individual concerned was pollen-sterile. Its ovule fertility has not been tested by cross-pollination. It arose by diploid parthenogenesis from the Java cane POJ 2725 ($2n = 106$) after pollination with *Imperata cylindrica*, as I have recorded elsewhere (Janaki-Ammal, 1940).
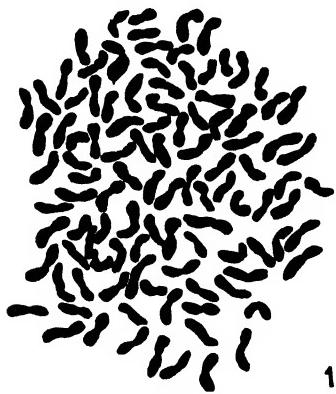
FIG. 1. Somatic metaphase in root-tip of spindle defective plant. $2n = 110$. ($\times 3,000$.)

METHODS

Root-tips were fixed in Allen's Bouin after immersion in ice, and stained in Heidenhein's iron-alum-haematoxylin (Fig. 1). Pollen mother-cells were fixed in 1:3 acetic alcohol and transferred to 70 per cent. alcohol, in which they were left for several months. Material thus preserved was stained in acetocarmine after immersion for a few minutes in acetic alcohol. Smears were made permanent according to McClintock's method.

OBSERVATIONS

Meiosis follows a uniform course until the first anaphase. There are a variable number of unpaired chromosomes and multivalents (Figs. 2–5), but these do not seem to hinder spindle development seriously. Even after this stage, in rare instances, telophase, second division (Fig. 6), and tetrad formation may succeed one another to give separate pollen grains with separate nuclei. These, however, when formed rapidly degenerate. Alternatively, the second division may be omitted, and irregular dyads formed which afterwards degenerate (Figs. 7–9).

The great majority of cells show abnormalities, which fall sharply into two classes. In both the first telophase nuclei lose contact with the spindle before a cell plate can be formed. The loss of contact is not merely a visible relationship in the fixed material. It is correlated with a loss of relationship in movement. The two courses that may follow are quite simple. In the one case, as in Figs. 11–15, the two nuclei leave the ends of the spindle and move towards one another, one on each side. They fail, however, to meet, and produce, when the spindle has disappeared, a single, ill-formed, binucleate pollen grain. In the second case, which is even more surprising (Figs. 16–21), the two nuclei move towards one another through the spindle. They meet inside the spindle, and having met fuse to form a single nucleus from which a single pollen grain develops.
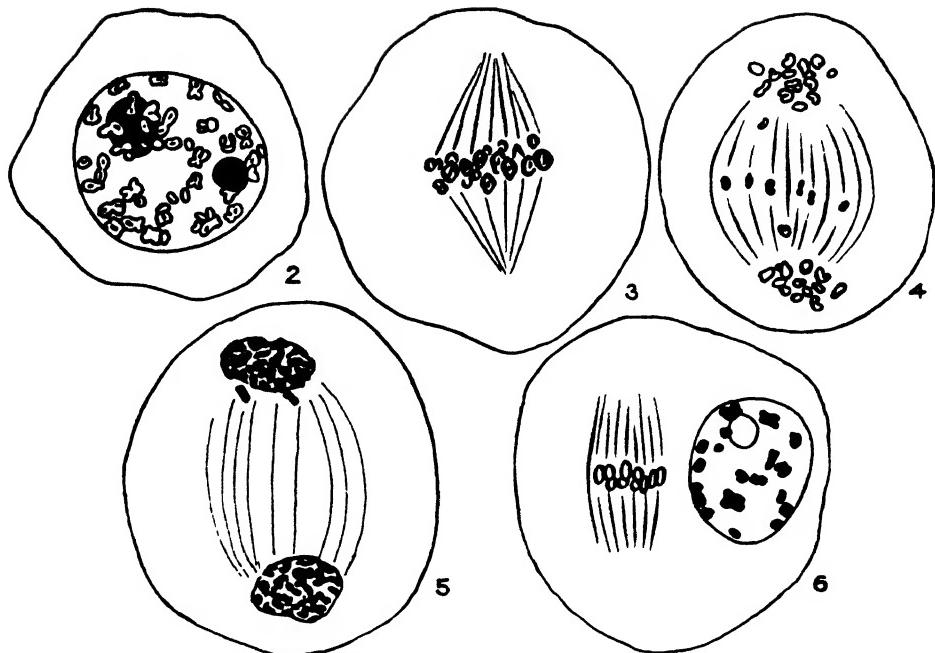
FIGS. 2–5. Diakinesis to first telophase in pollen mother cell.
FIG. 6. A rare case of second division (see text). (×1,200.)
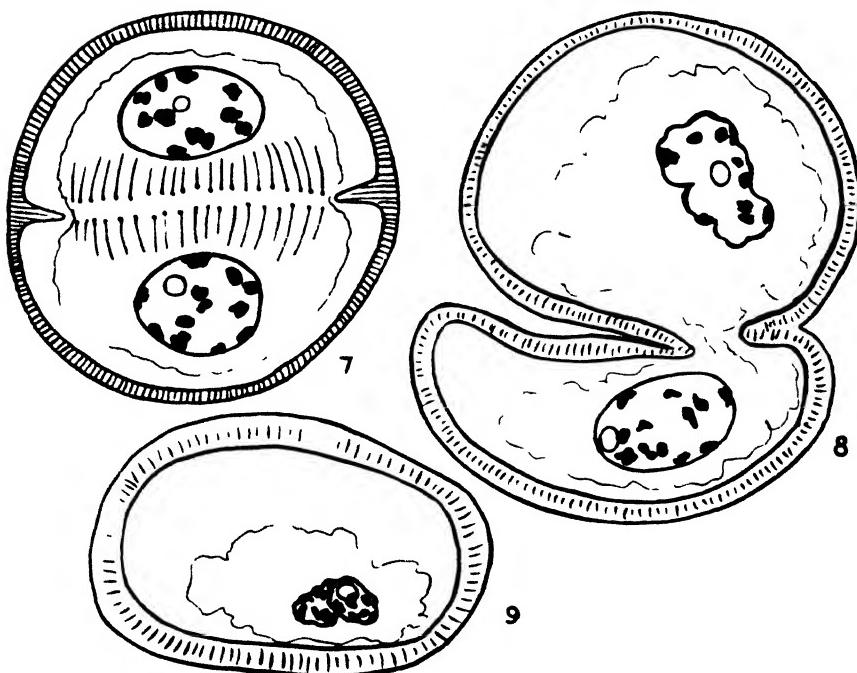
FIGS. 7–9. Formation of irregular dyad pollen grains through omission of second division.
(×1,200.)
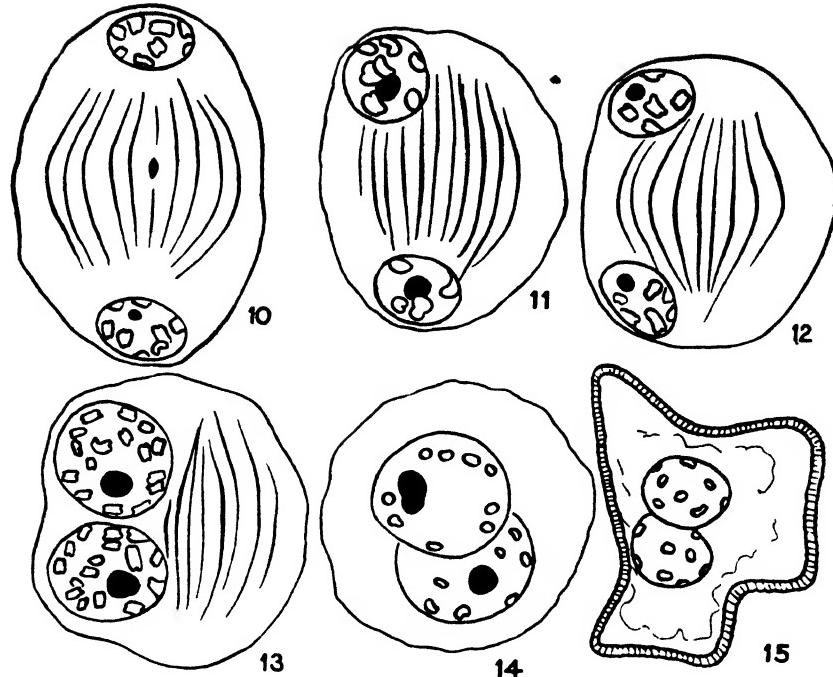
FIGS. 10–15. First telophase to formation of binucleate pollen grains. (× 1,200.)
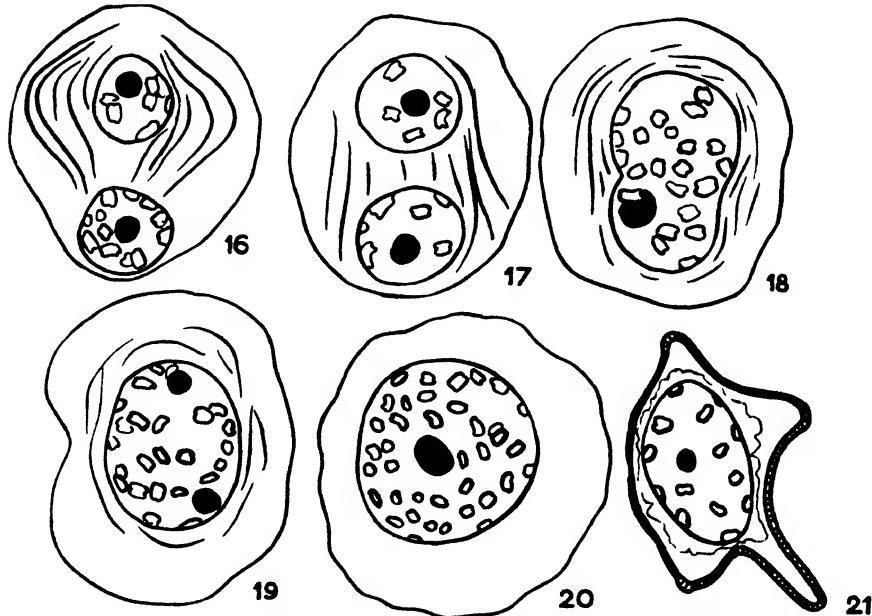
FIGS. 16–21. Formation of uninucleate pollen grains by fusion of the first telophase nuclei.
(× 1,200.)

What is significant about this behaviour is that nuclei, which are distinguished by the accidents of their position and movement, in one course of development fuse and in another fail to do so. They fuse when they are within the spindle; they fail to fuse when they are within the cytoplasm. A second point of interest is the loss of contact of nucleus and spindle, which recalls the absence of contact of the second division in the Lolium derivative. This loss of contact is of course associated with the loss of function in the deposition of the cell plate. We should therefore describe the spindles, for all their apparent structure and strength, as dead spindles.

SUMMARY

Sterility in a parthenogenetically derived seedling of the sugar-cane POJ 2725 is due to inactivity of the spindle after the first telophase.

The two nuclei formed either move down the sides of the spindle or pass through it. In the latter case a restitution nucleus results.

In rare cases tetrad and dyad grains are formed; these degenerate.

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Studies in Tropical Fruits

X. Preliminary Observations on Transpiration during Ripening

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With twelve Figures in the Text

I. INTRODUCTION

THE loss of water as vapour from plants has been the subject of very considerable study in attempts to determine the nature and mechanism of the control exercised by the different plant organs. In leaves, which are chiefly involved in transpiration, attention has been directed to determinations of cuticular and stomatal water loss and to the effect of the various factors constituting the external environment, a diversity of methods being used. In fruits attention has largely been concentrated on the loss of water of the fruit on the tree in relation to the pathological problem of fruit fall, or to loss during storage in relation to undesirable shrivelling. In few instances have observations extended over prolonged periods.

The water relations in a detached plant organ involve consideration of (*a*) water-loss to the external environment, (*b*) water-gain from metabolic sources, (*c*) the effects of changes in the composition and physical state of substances within the different tissues, and (*d*) changes in resistance to the movement of gases and water vapour through the intercellular spaces. The relations must therefore be of a complex nature.

In these studies on tropical fruits measurements of water loss during ripening have been made in conjunction with measurements of respiration. Since the water content of a tissue affects the resistance of that tissue to the movement of gases, water relations may be regarded as playing a part in all aspects of metabolism involving gaseous exchanges.

Fruits of different surface/volume ratio, structure, and succulence may be expected to show considerable differences in their transpiration rates during ripening. From the preliminary data submitted in this paper it will be seen that such measurements of the trend in rate of water loss during ripening show changes no less well-defined or characteristic than those of the accompanying respiration and other metabolic processes. In addition to these general features preliminary evidence has been obtained which indicates that the distribution of water in the tissues is subject to changes related to the chemical and physical changes undergone.

In so far as fruits are capable of taking up fluid when immersed in water or in aqueous solutions, the role of water in fruits has an interest which is not necessarily confined to fruit in storage. The development of latent fungal infections (Leonard, 1936; Wardlaw and Leonard, 1936a) at definite stages in the storage life of fruits calls for examination, among other aspects of metabolism, of the humidity conditions of the tissues concerned.

The curve of rate of respiration of a plant organ obtained by measurement of the carbon dioxide liberated at the surface is 'a statistical curve of the distribution of states of greater or less activity amongst the cell population' (Blackman, 1924). This may well be extended to include the liberation of all gases, including water vapour, and also of the output of heat by the plant organ; all of these are statistically representative of the whole cell population and subject to the interactions between cells and tissues, and together indicate the metabolic activity of the organ. On the other hand, the curves obtained from successive observations of the concentrations of the internal gases, from manometric records or from temperature observations such as have been given in earlier contributions in this series (Wardlaw and Leonard, 1936a, 1938, 1940), afford data of conditions within a plant organ which are representative of a cell population whose extent around the point of observation is unknown, and which, furthermore, may vary during the course of an experiment owing to changes in tissue resistance. Such relatively localized observations, while of great use in the investigation of regional changes, may not, therefore, be directly referable either in time or degree to changes occurring in the plant organ as a whole. In fruits with a large internal cavity, such as the papaw, the observations may be taken as a statistical representation of the environmental conditions common to all the cells contiguous to this cavity.

Some aspects of respiration in relation to surface effects have been discussed by Steward, Wright, and Berry (1932). They point out that the superficial cells (of potato slices) exhibit marked effects not shown by the cells of the

tissue mass as a whole, carbon dioxide and oxygen concentrations within the tissue being important in relation to the respiration of the cells at different depths from the surface.

In an earlier paper (Wardlaw and Leonard, 1936*a*) reference was made to the importance of the surface/volume relationship during the development of fruits. Preliminary experiments on the respiration and transpiration of developing, immature bananas indicate that very rapid changes take place on removal from the plant. While such studies are of considerable value the necessity for concentration of interest on fruits at about the stage of commercial harvesting precludes their being followed up at present.

Several issues of practical importance are involved in a study of the water loss from fruit in storage: (i) a considerable proportion of the heat removed during the initial cooling of a cargo of fruit is effected as latent heat of vaporization; (ii) at a given storage temperature the proportion of the total heat taken up by the refrigerating plant as latent heat of vaporization is dependent upon the nature and condition of the fruit stored and upon the humidity of the storage-room air. The design and equipment of storage chambers where humidity as well as temperature are to be controlled thus involves consideration of the rate of transpiration of the stored fruit. Some aspects of humidity control in commercial storage have been discussed by A. J. M. Smith (1932) with the comment that, in order to reduce the humidity from the value characteristic of any fruit in storage, high rates of mass-velocity of air movement are necessary.

The present paper is of a preliminary nature only, the full exploration of the subject calling for a scheme of work not less extensive than that devoted to respiration. The results are confined to the demonstration that during ripening the trends of water loss give curves of characteristic shape. These may be divided, on the basis of the data so far obtained, into three types: (i) those showing, after harvesting, an initial fall in transpiration rate, followed by a subsequent steady level, (ii) those showing an initial fall to a steady level followed later by a continuous rise during ripening, and (iii) those whose trend follows closely that of the respiration rate, i.e. an initial fall to a preclimacteric level, a rise during the climacteric and a fall to a new level which is higher than the preclimacteric level. These have been correlated (*a*) with some of the readily recognizable changes which take place during ripening (coloration of skin, softening, &c.), (*b*) in the papaw, with the concentration of internal (cavity) gases, and (*c*) in the banana, with the curve of respiration rate.

II. METHODS AND MATERIALS

Total loss of weight has been the sole method of estimating the transpiration rate of fruits in storage in the present study, and has been expressed as gm. per kg. of fruit per hour, based on the mean weight between successive observations, i.e. relative rate of loss, since the intervals were of variable

length and the change in weight considerable. The weight method involves measurement of the total loss due to gases and vapours liberated, but since even in a saturated atmosphere the total loss is three times that of the amount of carbon dioxide evolved, and therefore about eleven times that of the carbon lost, the error involved, especially at lower humidities, is slight.

In exploratory experiments with fruits enclosed in containers using a 'draw-through' system, absorption in calcium chloride U-tubes, of the water liberated, followed by absorption of the carbon dioxide, was attempted. This method suffers from serious disadvantages: (i) either dry air must be admitted at the inlet side of the container which involves considerable desiccation of the fruit, or a 'split stream' method must be used to allow of correction for the water admitted on the inlet side; (ii) frequent clogging of the absorbing tube appears unavoidable.

The fruits were stored in an insulated storage room fully exposed to the air (except with those whose respiration rate was to be determined) and removed for weighing to an adjacent storage room maintained at the same temperature but at lower humidity; the minimum possible time was occupied in this operation. In occasional instances, where a slight error in a weighing resulted in an apparent rapid rise in transpiration rate followed by a fall (or vice versa) between successive weighings, the intermediate weighing has been neglected and the rate of loss in weight calculated over the whole period.

The equipment for providing controlled temperature and humidity has been described elsewhere (Wardlaw, Leonard, and Watts, 1938); the environmental records for individual experiments were obtained by means of an open scale sensitive bimetallic thermograph, with point observations by means of a mercury thermometer divided to $0.2°$ F., and a hair hygrograph standardized against a wet and dry bulb hygrometer. The air delivery to the storage room was such as to give a rate of change once every four minutes; with a uniform distribution of air speed across the room this would give 3–4 ft. per min. When any considerable quantity of fruit was present in the room it was necessary to 'blow out' the room once every twenty-four hours to avoid an undesirable accumulation of carbon dioxide and volatile substances.

In experiments with papaws analyses of the internal atmospheres of the cavity were made during ripening, using the method described previously (Wardlaw and Leonard, 1936a). The sampling tubes, however, were inserted by boring a hole at the stalk end instead of equatorially. This gives a more rigid arrangement which is advantageous when the fruit is being handled frequently.

Where respiration and transpiration rates of bananas were obtained, using the same 'fingers', these were removed from the respiration chambers, weighed and replaced, the 'draw-through' system being temporarily shut off. The details of the lay-out have been published in an earlier paper (Wardlaw and Leonard, 1939). The temperature and humidity records were from point observations of the mercury-in-glass thermometer inserted in the chamber and the paper hygrometer at the outlet end.

The majority of the experiments were carried out at high temperatures (84°–88° F.) and high humidities (e.g. 85 per cent. R.H.). This affords several advantages from the point of view of experimentation: (1) considerable losses in weight occur during a relatively short time and irregularities in the curves of loss in weight due to slight errors in weighing assume less importance; (2) the whole course of ripening occupies a short time (10 to 14 days for bananas) and repetition or amplification of records can be obtained with the minimum delay; (3) the conditions constitute an approximation to the ripening of the fruits in their normal environments in the tropics. Fruits in refrigerated storage show essentially a prolongation of ripening though diversion of the trends of metabolism may occur. Trends of transpiration rate of similar type have subsequently been obtained for bananas at 68° F. (20° C.).

In every instance (except certain tomatoes, q.v.) fruit of sufficient maturity was taken to give what may be described from general experience as 'normal' ripening.

III. The Trend of Transpiration Rates during Ripening

Type I. Tomato.

Tomatoes of the variety Marglobe were picked about full grown, pale green, and free from 'sun-cracks' at the calyx which was left in position, the cut end of the stalk being sealed with vaseline. Weighings were made at regular intervals; the relative transpiration rates for five fruits are given in Fig. 1. The temperature and relative humidity records in the storage room are given at the top of the figure; a mean temperature of 88° F. obtained during the first ten days, rising to 88·5° F. later with a relative humidity of 85 per cent. It will be seen that the transpiration shows an initial, relatively slow fall to a steady level, and that no perceptible change in transpiration occurred during the process of coloration of the skin which accompanies ripening of the internal tissues (Wardlaw and Leonard, 1936a) It must be noted that fruits I, II, and III ripened normally, i.e. there was a steady progression of colour from pale green to yellow, orange, and red accompanied by softening of the tissue, whereas IV and V, which were smaller fruits, remained relatively hard to the end of the storage period and developed an abnormal pink skin. All five, however, showed a similar trend of transpiration rate.

For completeness additional data are tabulated below.

TABLE I

Fruit No.	Initial weight. (gm.)	Final weight. (gm.)	Loss (%) of initial weight).	Vol. (c.c.)	Final condition.
I	110·15	97·59	11·40	102	Red, orange at calyx, wrinkled, no rotting.
II	82·90	76·81	7·35	85	Red, orange at calyx, considerable internal rotting.
III	77·43	69·04	10·83	70	As I.
IV	77·43	68·42	11·63	80	Orange, yellow at calyx, no rotting.
V	73·85	66·43	10·05	75	As IV.
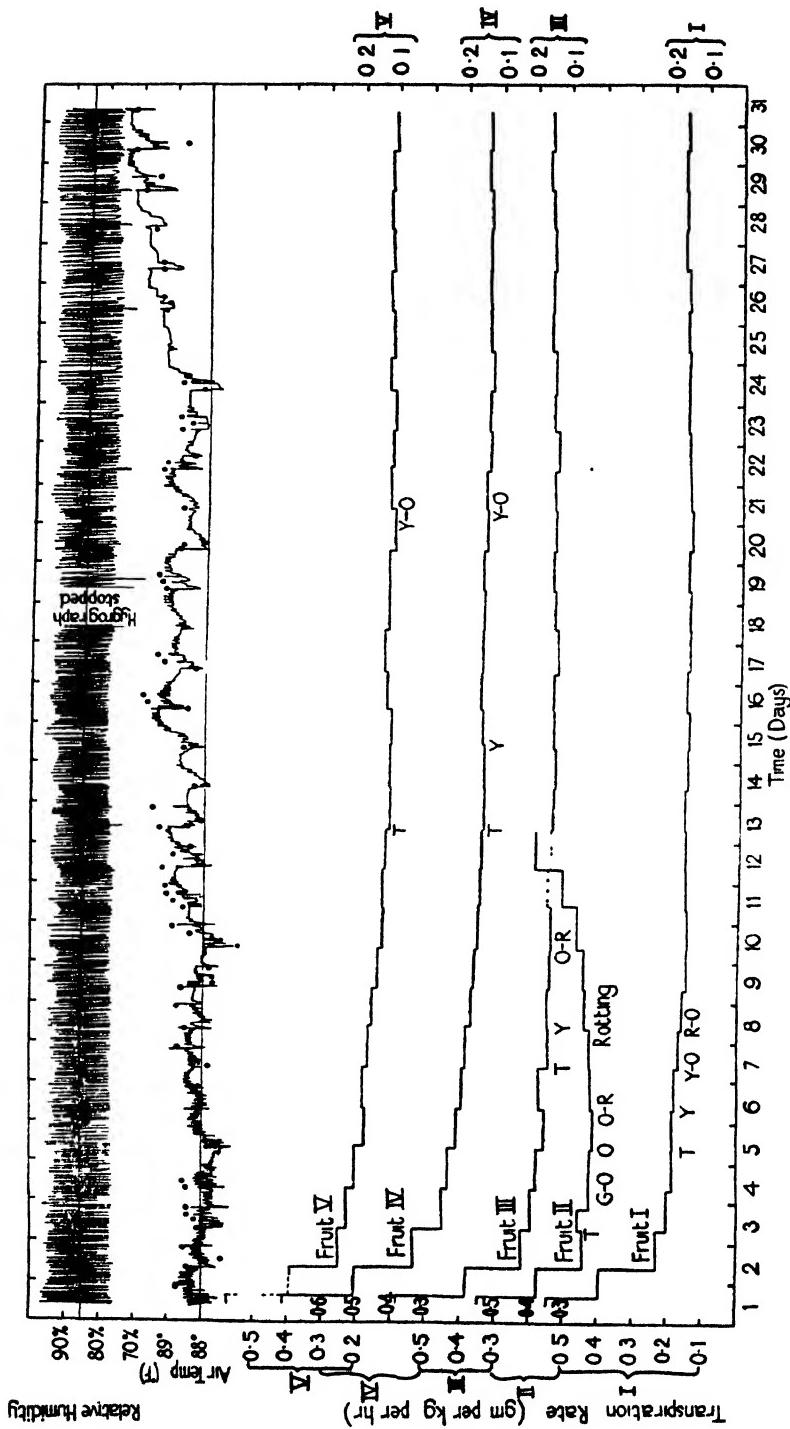
FIG. 1. Rates of transpiration of five Marglobe tomatoes during ripening at 88°–88·5° F. and 85 per cent. R.H. Observations on skin coloration are indicated: G, green, T, first trace of colour, Y, yellow, O, orange, R, red. Air temperature records (° F.) from a bimetallic thermograph are given together with point observations by mercury-in-glass thermometer, and relative humidity by hair hygrograph.

No data on respiration rates of tomatoes are submitted here, but preliminary experiments at tropical temperatures have shown that there is a climacteric rise of respiration rate accompanying the change in skin colour, followed by a fall. Such a rise and fall have also been recorded by Gustafson (1929), Walford (1938), Singh and Mathur (1936, 1939), and others. Gustafson found in general that there is a climacteric rise in respiration rate accompanying the colour change; Walford obtained different types of respiration records with summer and winter grown fruits, which he classes as 'conventional' or 'anomalous'; while Singh and Mathur (1939) suggest that it is significant that certain tomatoes when picked green showed no evidence of a climacteric peak, though undergoing the normal colour change.

The very considerable storage period at high temperatures possible with tropically-grown tomatoes is of interest in connexion with the differences in behaviour between such fruit and those grown in temperate countries, which in turn show differences in storage qualities between summer and autumn grown fruit. It would appear that considerable differences may exist in the physiological age and behaviour of this fruit resulting from different environments during growth.

Type II. Papaw.

A different curve of transpiration rate is found during the ripening of this fruit. As has been stated elsewhere (Wardlaw, Leonard, and Baker, 1934) papaws must not be harvested until a trace of colour has appeared in the skin if normal ripening is to ensue, and if a full orange skin is to result, a considerable amount of colour must be present at picking. Also, the spread of yellow colour of the skin marks the rise in the climacteric liberation of carbon dioxide, a full yellow skin corresponding to the peak value (Wardlaw and Leonard, 1936a). To determine, therefore, the steady rate of respiration previous to the climacteric either a synthetic curve must be constructed by the use of data from full-grown but green fruit for the pre-climacteric stage, and, for the later part, from other fruit picked with the first trace of yellow colour of the skin; alternatively the lower, pre-climacteric respiration rate must be established over a relatively brief period by picking a papaw with the very first trace of yellow skin coloration. These considerations, of course, apply equally to any measurements which are to be made during ripening.

As a preliminary attempt to establish the relationship between the transpiration rate and respiration phenomena papaws of the 'Country' variety, a small, sub-spherical, slightly furrowed variety, were picked at various stages of maturity as indicated by their position on the plant, size, and skin coloration. Their internal gas concentrations and rates of transpiration were determined at intervals as previously described. The temperature and humidity records are those for the first eleven and twelve days of Fig. 1. The records for two typical fruits are given as graphs in Figs. 2 and 3 and the following additional data are appended.
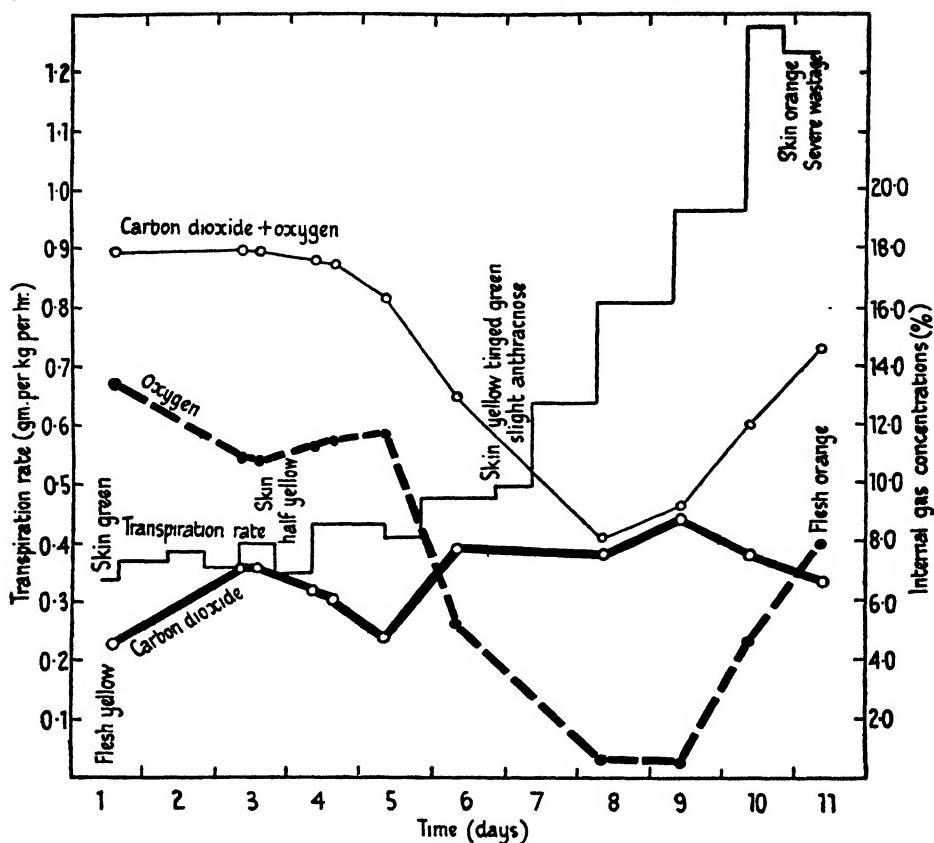
FIG. 2. Rate of transpiration of a papaw ('Country') during ripening at 88° F. and 85 per cent. R.H. together with internal concentrations (per cent.) of oxygen, carbon dioxide, and oxygen *plus* carbon dioxide. Observations on skin and flesh colour and on the incidence of anthracnose spots are given.

TABLE II

Fruit.	Initial weight. (gm.)	Final weight. (gm.)	Loss (% of initial weight).	Vol. (c.c.)	Cavity vol. (c.c.)	Skin. Initial.*	Final.	Flesh. Initial.*	Final.
Fig. 2	745·5	644·5	13·55	716	50	Pale green.	Orange, about half covered with mycelium.	Yellow.	Orange, slight internal fungus, seeds black.
Fig. 3	601·0	531·0	11·65	975	28	Dark green.	Dingy orange flecked with green, considerable fungal wastage.	White to just yellow next cavity.	Orange and 'glassy' no internal fungus, seeds black.

* The initial flesh colour is observed in the plug of tissue withdrawn from the hole for insertion of the sampling tube.

In this variety and under the conditions of the experiment (temperature, &c.) the climacteric is seen to be marked by a rise and slight fall in the internal carbon dioxide concentration following the attainment of full yellow colour of the skin, instead of the steady rise observed previously in larger, Porto Rico papaws (Wardlaw and Leonard, 1936*a*). This is analogous to the results
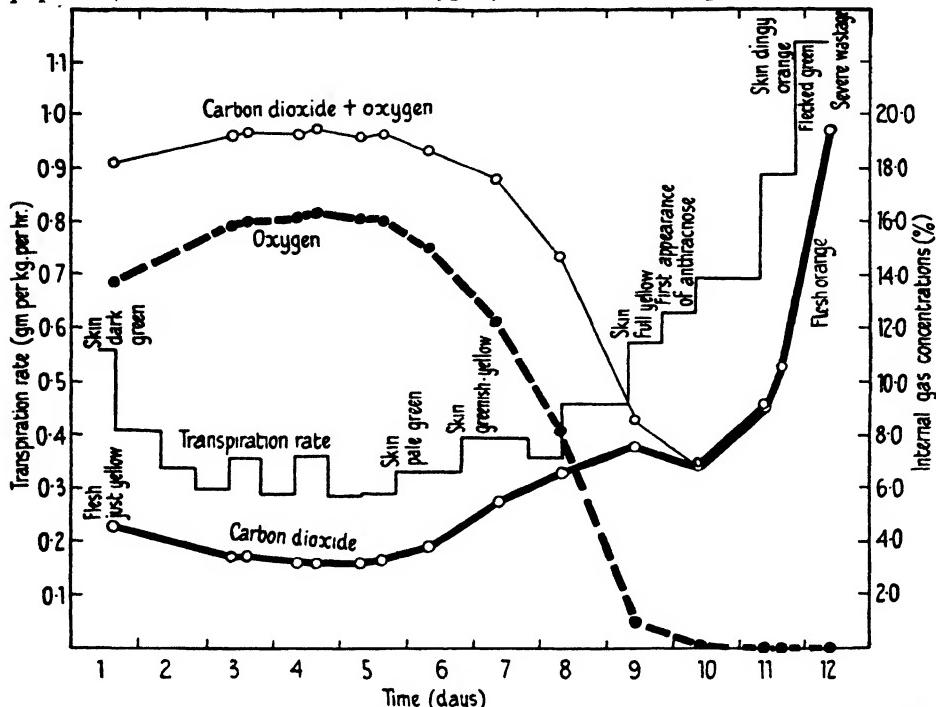
FIG. 3. Rate of transpiration of a papaw ('Country') during ripening at 88° F. and 85 per cent. R.H. together with internal concentrations (per cent.) of oxygen, carbon dioxide, and oxygen *plus* carbon dioxide. Observations on skin and flesh colour and on the incidence of anthracnose spots are given.

obtained in subsequent experiments with bananas (Wardlaw and Leonard, 1940). The transpiration rates, however, show a steady rise during the spread of skin colour, increasing to very high values with the onset of skin disruption by anthracnose. To this progressive regional ripening of the skin (Wardlaw and Leonard, 1936*a*), in which the furrows colour before the ridges, may be attributed the continuous rise in transpiration rate. Investigation of such regional ripening by determination of transpiration rates at different points on the fruit (e.g. by cobalt chloride paper) would be of interest. Similarly, the steady rise in the internal concentration of carbon dioxide may be attributed to the progressive ripening of the tissue abutting on the internal cavity.

Type III.

A third type of transpiration trend during ripening was found to occur in mangoes and bananas.

Mango. Julie mangoes were picked at the stages described as 'shoulders level' or 'shoulders raised' (Wardlaw and Leonard, 1936). They were stored alongside the tomatoes described above and the storage-room temperature and humidity records are therefore those for the first thirteen days of Fig. 1. Weighings were made at regular intervals and the transpiration rates of five fruits are given in Fig. 4. It will be seen that there is a marked rise in the rate during the ripening process after a steady, lower level or an initially declining rate, the ripe fruit then maintaining a higher level for two or three days prior to the final rapid rise on disruption of the surface by fungal rotting (chiefly due to anthracnose, *Colletotrichum gloeosporioides*). The following additional information is tabulated:

TABLE III

Fruit No.	Develop-mental condition.	Initial weight. (gm.)	Final weight. (gm.)	Loss (% of initial weight).	Vol. (c.c.)	Weight of stone. (gm.)
I	'Shoulders raised'	348·56	258·91	25·61	250	29·45
II	,,	345·11	273·73	20·63	270	25·32
III	,,	295·01	216·04	26·77	210	17·54
IV	'Shoulders level'	354·17	274·49	22·50	268	22·73
V	,,	331·67	240·50	27·49	230	27·00

All the fruit reached normal ripeness and overripeness, the flesh becoming full orange, watery and alcoholic, and the skin showing a considerable spread of anthracnose spots, but no penetration into the flesh was observed except in fruit III. The spread of anthracnose is preceded by a slight darkening of the skin below the epidermis. Full yellow coloration of the skin occurred only in fruits II and III, those of 'shoulders level' remaining green and showing considerable wrinkling. The 'sprung' condition, when the flesh yields to slight pressure, is fairly readily recognizable and coincides with the first stage of 'eating ripeness'. The curves are annotated to show the ripening changes observed.

Cheema, Karmarkar, and Joshi (1939) give values for loss in weight of three varieties of mangoes at 68° F. and state that there was no sudden change in rate of loss at the time when the fruit ripened. Their observations, however, appear to have been made at rather wide intervals and they give only a curve of percentage loss in weight against time, based apparently upon the mean loss in weight of twelve fruits.

No data on respiration rates are submitted for mangoes. Singh, Seshagiri, and Gupta (1937) found that Langra mangoes from cold storage at 8° C. gave a fairly steady rate of respiration for the first thirty hours followed by a gradual fall; this was followed by a further steady, level phase and then a continuous decline. Elsewhere (1937a) they give data showing a rise and fall in respiration rate of full grown Langra mangoes, direct from the tree, during
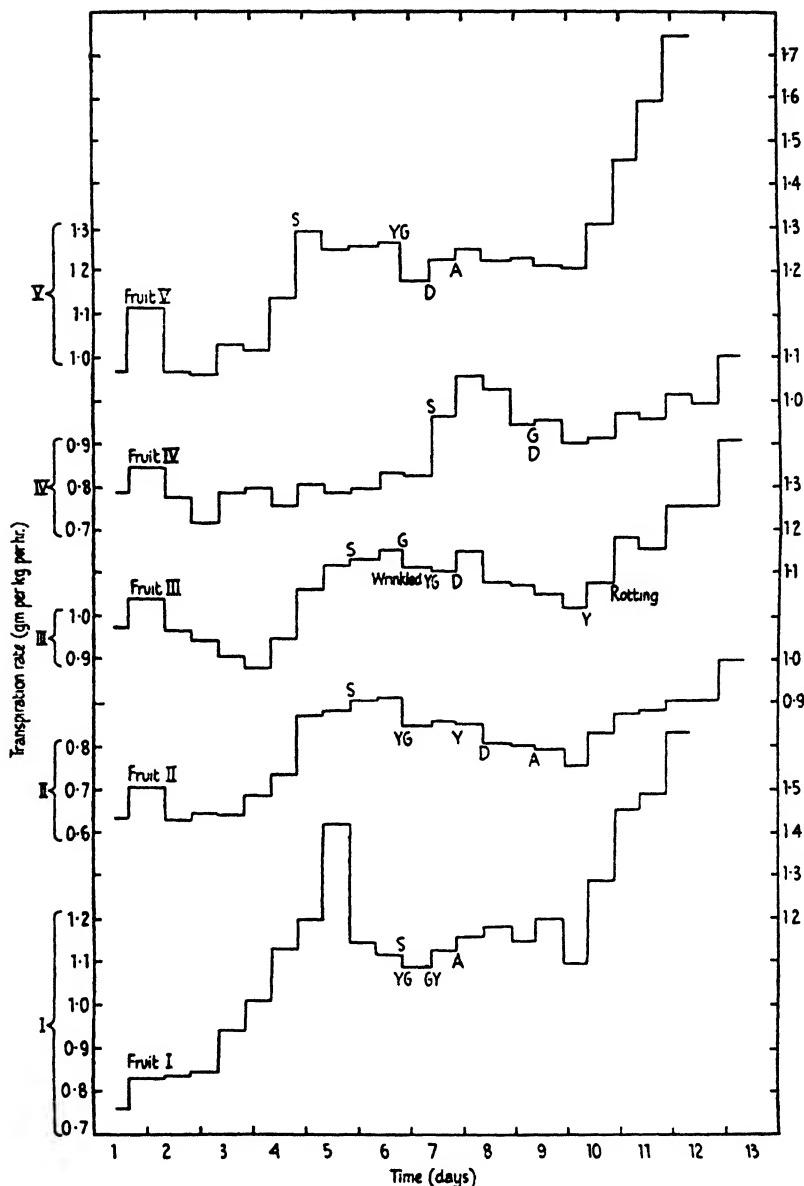
FIG. 4. Rates of transpiration of five Julie mangoes during ripening at 88°–88·5° F. and 85 per cent. R.H. The times of change in skin coloration, attainment of the 'sprung' condition, and the incidence of anthracnose spotting are indicated: G, green, YG, yellowish-green, GY, greenish-yellow, Y, full yellow, D, skin darkening, A, first appearance of anthracnose spots, S, 'sprung'.

a stage they term 'senescent'; they suggest that during the previous 'climacteric' stage the continuous decline in respiration rate is due to the developing endocarp and mesocarp acting as obstructions to gaseous exchange.

Banana. In general the trend of transpiration rate of individual bananas

during ripening resembles that for mangoes, that is, there is an initial steady, relatively low rate for the green, unripe fruit, followed by a rapid rise during the early part of the ripening process. The 'ripe' and 'overripe' fruit shows
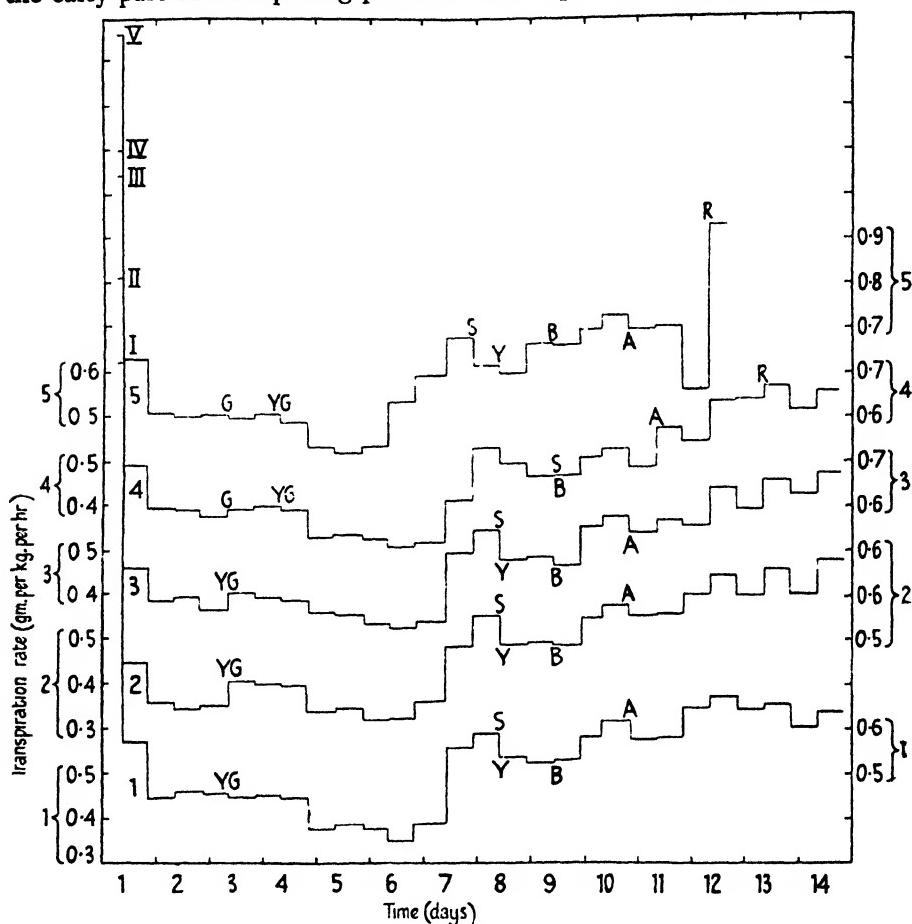
FIG. 5. Rates of transpiration of five fingers from the centre of the upper (proximal) row of fingers of the fourth hand of a 'heavy ¾-full' bunch of Gros Michel bananas during ripening at 84·5° F. and 85 per cent. R.H.   The times of skin coloration, attainment of the 'sprung' condition, and the incidence of anthracnose spotting are indicated: G, green, YG, yellowish-green, Y, full yellow, S, 'sprung', B, brown mottling, A, first appearance of anthracnose spotting, R, rotting.

a fairly steady but usually slowly rising rate until the skin has become completely brown and serious fungal wastage takes place, when a final rapid rise may occur. This is exemplified in Fig. 5 which gives transpiration rates for five fingers from the centre of the upper (proximal) row of the fourth hand of a ten-hand[1] 'heavy ¾-full' bunch of Gros Michel bananas. The fingers were

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[1] The small eleventh hand would not be included in a commercial 'count'. An explanation of the terms used to describe commercial bunches of bananas is given elsewhere (Wardlaw, Leonard, and Barnell, 1939).

cut from the subtending cushion immediately on receipt, which was some six hours after harvesting; they were allowed to exude latex until this stopped, when the cut was wiped with 0·2 per cent. mercuric chloride solution and smeared lightly with vaseline. They were weighed at regular intervals of twelve hours during storage at 84·5° F. and 85 per cent. R.H. The temperature and humidity records for this experiment are those given in Fig. 6. The very considerable uniformity in the type of transpiration curve of the different individual fingers, in the actual rates of transpiration, and in the incidence of the climacteric will be noticed; the climacteric rise occurred slightly earlier in fruit No. 5 than in the other four. The times are given at which yellow coloration of the skin first appeared, the full yellow skin and the 'sprung' condition were attained, the brown mottling of the skin was just seen, and anthracnose spots and subsequent rotting first appeared.

Fig. 6 gives transpiration rates for upper and lower row fingers of this hand. The values for the upper row are those for the mean rate of fingers 1 to 4 of Fig. 5 and for five lower row fingers; the higher rate of transpiration of the lower row fingers is noticeable. It has been observed (Wardlaw, Leonard, and Barnell, 1939) that the pulp/skin weight ratio of fingers in the lower row in the green condition is consistently greater than that of fingers in the upper row, even for fingers of approximately equal weight in the two rows. The ratio is also seen to be greater in the lower row fingers in the overripe condition.

Table IV gives additional data for the fingers of Figs. 5 and 6.

TABLE IV

Fruit.	Fruit No.	Initial weight. (gm.)	Final weight. (gm.)	Loss (%o of initial weight).	Final weight of skin. (gm.)	Pulp/ Skin weight ratio.	Final condition.
Upper row	1	154·86	130·40	15·79	25·75	4·07	Skin brown, pulp watery, no fungus.
	2	159·19	136·04	14·54	26·20	4·19	As 1.
	3	161·08	137·55	14·61	28·15	3·89	As 1.
	4	163·44	140·08	14·30	29·07	3·82	Skin brown, slight stem-end rot.
	5	178·94	151·54	15·32	33·62	3·41	Skin brown, stem-end rot spreading into pulp.
Lower row	6	130·01	106·65	17·97	20·29	4·26	Skin brown, slight stem- and distal-end rot, pulp watery, no fungus.
	7	134·41	110·22	18·00	20·29	4·43	As 6 but slight internal fungus.
	8	139·02	116·92	15·90	21·82	4·36	As 6 but considerable internal fungus.
	9	139·22	116·47	16·34	21·63	4·38	As 6.
	10	142·40	116·98	17·84	21·35	4·48	As 6.

The rates of transpiration of the other hands (I–III and V–XI) and of the cylindrical stem at the proximal end of this bunch of bananas were also determined, the cut ends of the stem in each case being treated in the same way as were the individual fingers of the fourth hand. A balance of suitable accuracy was not available, with the result that irregularities are introduced into the
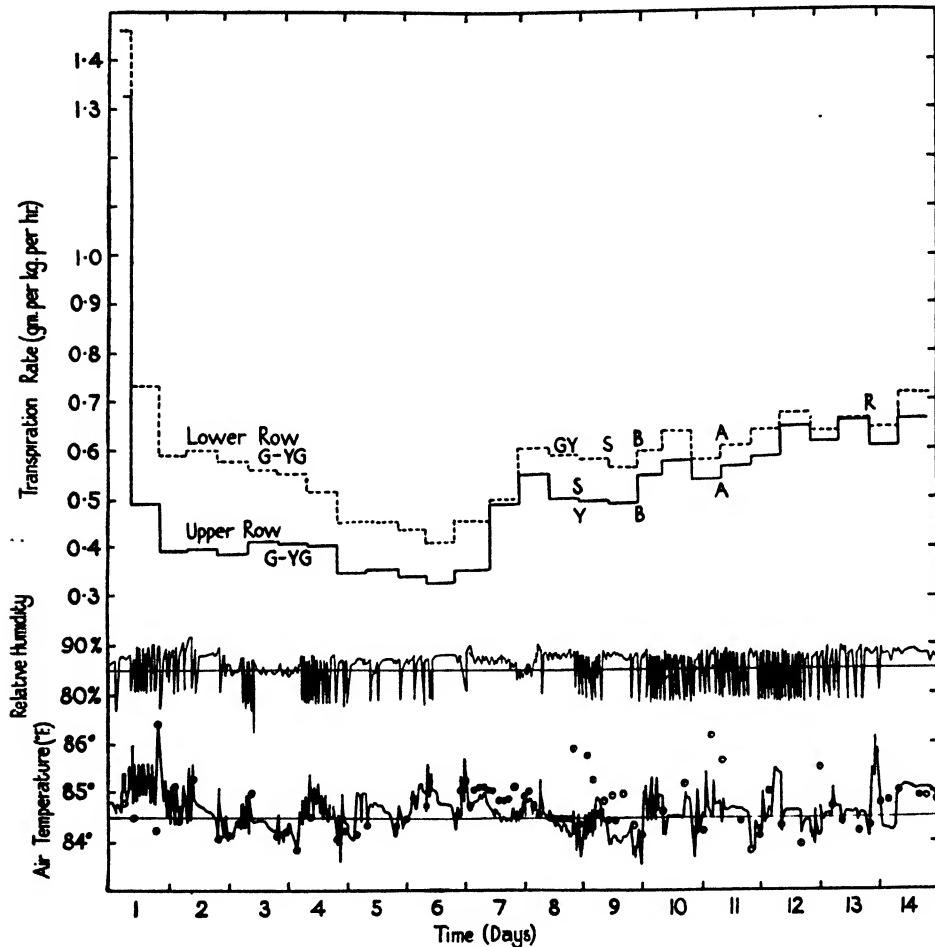
FIG. 6. Mean rate of transpiration of four fingers (1 to 4 of Fig. 5) from the centre of the upper row of fingers of the fourth hand of a 'heavy ¾-full' bunch of Gros Michel bananas, and of five fingers of the lower row of the same hand during ripening at 84·5° F. and 85 per cent. R.H. Skin colour, &c., notations as Fig. 5. Air temperature record (° F.) from a bimetallic thermograph together with point observations by mercury thermometer; relative humidity by hair hygrograph.

curve of transpiration rate due to inaccuracies in weighing; nevertheless, the general trend of transpiration is evident in Fig. 7, which gives the data for the piece of stem and for alternate hands. The temperature and humidity records are, again, those of Fig. 6; the skin colour change, &c., are given as in previous
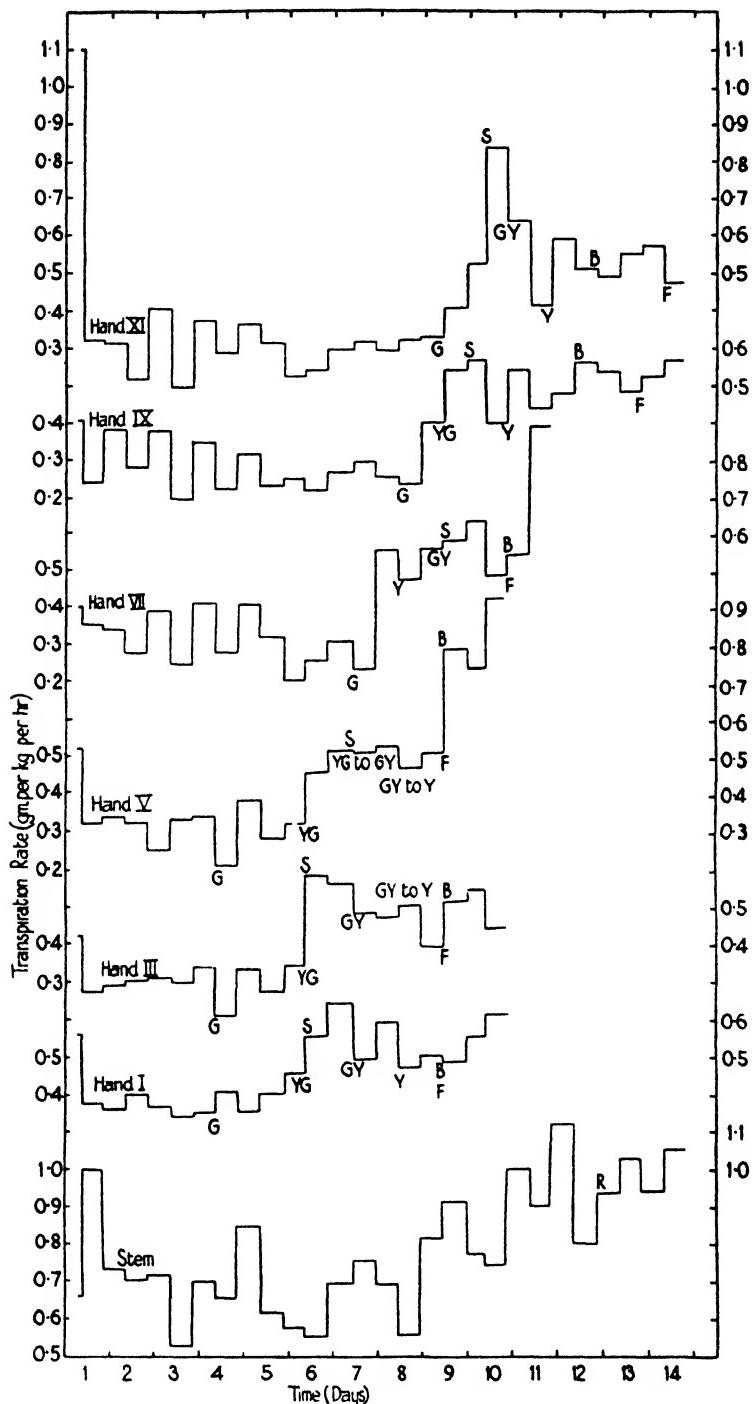
FIG. 7. Rates of transpiration of stem and of alternate hands of a heavy '⅔-full' bunch of Gros Michel bananas during ripening at 84·5° F. and 85 per cent. R.H. Notations for skin colour, &c., as in Fig. 5. F indicates fingers falling from hand.

TABLE V

Hand No.		Initial weight. (kg.)	Final weight. (kg.)	Loss (% of initial weight).	Upper row.			Lower row.		
					Final weight of single finger. (gm.)	Weight of skin. (gm.)	Pulp/ Skin weight ratio.	Final weight of single finger. (gm.)	Weight of skin. (gm.)	Pulp/ Skin weight ratio.
Stem piece	.	0·6745	0·5215	22·69	—	—	—	—	—	—
I (proximal)	.	3·952	3·5542	10·07	163·97	41·86	2·92	159·64	40·79	2·92
III	.	3·162	2·8865	8·71	165·00	41·46	2·98	158·05	41·62	2·80
V	.	2·9435	2·357	8·18	137·38	37·00	2·81	140·85	34·43	3·09
VII	.	2·194	1·972	10·12	118·27	34·13	2·47	103·35	27·76	2·72
XI	.	2·170	1·917	11·66	118·26	29·76	2·97	116·67	27·61	3·23
XI (distal)	.	1·619	1·4199	12·30	79·26	22·62	2·50	101·83	24·90	3·09
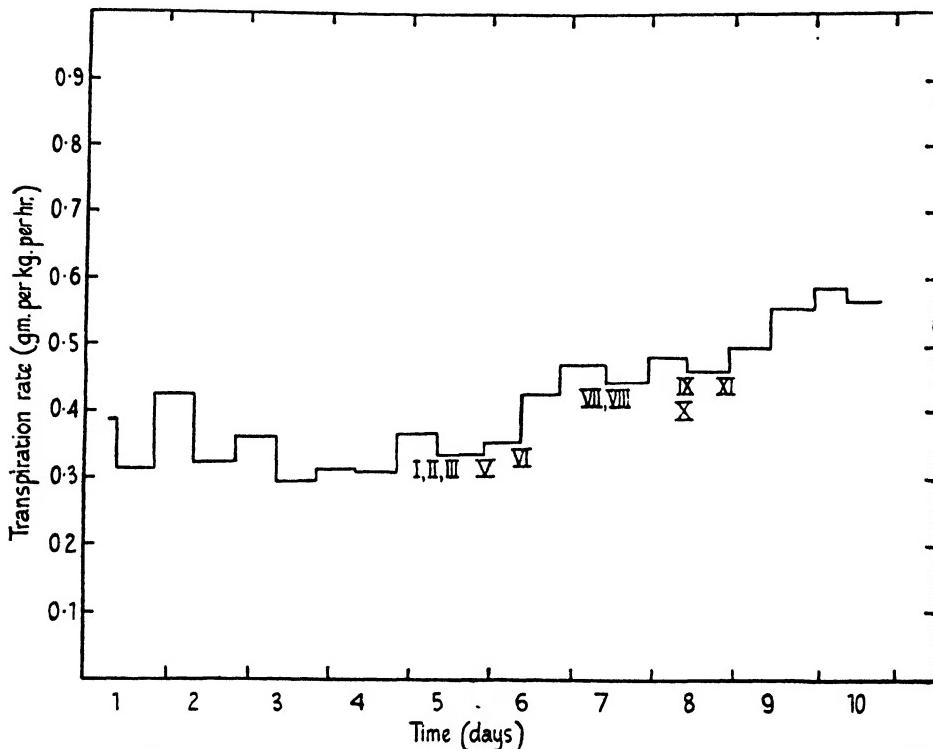
FIG. 8. Rate of transpiration of a ten-hand, heavy '⅞-full' bunch of Gros Michel bananas, calculated by summation of the losses in weight of individual hands (omitting No. IV) during ripening at 84·5° F. and 85 per cent. R.H. The times at which the rate of transpiration of the individual hands began to rise from the preclimacteric steady rate are indicated by roman numerals.

figures. Records of the individual hands were brought to an end by the detachment of the fingers under their own weight when overripe. Table V gives data for these hands. The trend of transpiration rate for fingers united in hands is seen to resemble that of individual fingers, and the acropetal progression of ripening of the hands along the bunch is evident. Comparison of Figs. 6 and 7 shows that detached fingers have a slightly higher rate of transpiration than similar fingers when attached to a hand: the transpiration rate of the stem is considerably higher than that of the hands. Data are also

available indicating that in the preclimacteric stage the transpiration rate of hands is higher than that of whole bunches. In view of the important effects of the rate of transpiration and of water content on rate of respiration, differ-ences in respiration rate between fingers, hands, and bunches may be ex-pected. It is anticipated that respiration data on the two last will be obtained shortly.

If the loss in weight of the individual hands (omitting No. IV) is summated and the rate of transpiration calculated for the whole bunch the transpiration curve of Fig. 8 results. Figures inserted alongside the curve indicate the points in time at which the rate of transpiration of the different hands started to rise from the initial steady rate. The general effect is to smooth out the climac-teric rise and fall to a value for the whole bunch which rises fairly steadily. A suitable balance was not available for weighing an intact bunch, but data obtained subsequently substantiated this type of transpiration trend for intact bunches. The type resembles that for the papaw and expresses the cumula-tive effect of the progressive increases in transpiration rate of the individual hands during ripening.

In Table IV data are given of the ratio of weight of pulp to weight of skin on termination of the records for fingers of the fourth hand, and in Table V for this ratio as determined from a single finger from the middle of each row of the different hands. The high degree of uniformity of this ratio for the fingers of each row of each hand has been the subject of comment elsewhere (Wardlaw, Leonard, and Barnell, 1939). It is seen that there is a fairly close agreement in the ratio as between the fingers from corresponding rows in different hands at final senescence.

Data on transpiration rates during ripening were obtained in a subsequent experiment using the hands from a ten-hand '¾-full' bunch of Gros Michel bananas (total weight on receipt 22·6 kg.). At the commencement of the experi-ment and at intervals during ripening one finger was removed from the upper row of each hand for determination of the pulp/skin ratio, the cut finger-stem was sterilized and smeared with vaseline and observations continued on the residue of the hand. The results for alternate hands are given in Fig. 9, pulp/skin ratios being superimposed on transpiration rates. Air temperature and humidity records are given in Fig. 10. The frequency at which weighings were made were based on the results obtained in the previous experiment so that the climacteric rise in transpiration rate is more clearly demonstrated. The times at which latex flow ceases after detaching the sample finger, of the appearance of honey colour about the placenta, of the attainment of the 'sprung' condition, together with observations on alterations in skin colour and the appearance of skin mottling and anthracnose (*Gloeosporium musarum*) spots, are given. A discussion of the changes observed in the tissues during ripening has been given elsewhere in relation to respiration (Wardlaw and Leonard, 1940). It will be seen that in all hands cessation of latex flow and the appearance of honey colour in the placenta coincided and occurred about
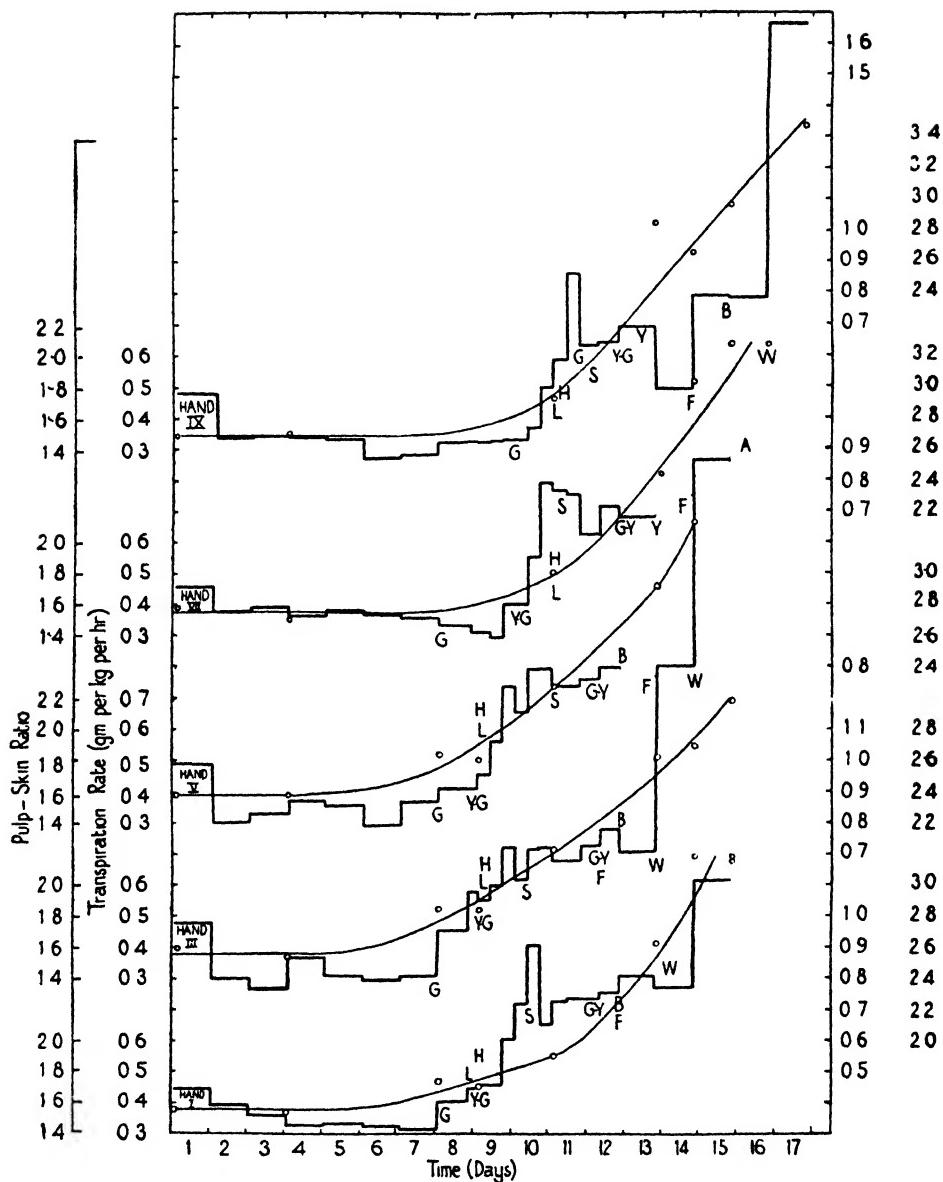
FIG. 9. Rates of transpiration of alternate hands of a ten-hand '¾-full' bunch of Gros Michel bananas during ripening at 85°–87° F. and 85 per cent. R.H.: and values of pulp/skin weight ratio of individual fingers from the upper row of each hand given as point observations (in circles) connected by a smoothed curve. Notation for skin colour, &c., as in Fig. 5. H, honey-coloured placenta, W, watery pulp, L, time at which latex flow last occurred on removal of sample finger.

half-way through the climacteric rise in transpiration rate, but that the development of yellow skin coloration was somewhat delayed in the more distal hands VII and IX.

A relatively steady pulp/skin ratio obtains until the occurrence of the climacteric rise in transpiration rate when rapid and considerable increases occur, reaching very high values relative to those in the unripe, green fruit. The skin is not only losing water to the surrounding air by transpiration but also to the pulp as a result of the increased suction pressure due to the increasing concentrations of sugar and other soluble substances in the pulp. Further discussion of this aspect of water movement is postponed.

Table VI contains additional data from this experiment.

TABLE VI

Hand No.	Initial weight. (kg.)	Percentage of total bunch weight.	Final loss (% of initial weight).
I	3·120	13·80	19·30
III	2·6935	11·92	23·57
V	2·066	9·14	16·78
VII	1·830	8·10	19·11
IX	1·854	8·20	21·24

Two fingers of the upper row of the fourth hand of this bunch were detached at the start of the experiment and their transpiration rates determined, weighings being made at more frequent intervals during the climacteric than previously, so that, as in the case of the hands, this phase is emphasized in the curves of Fig. 10, which gives temperature and humidity records.

Table VII gives additional data.

TABLE VII

Finger.	Initial weight. (gm.)	Final weight. (gm.)	Loss (% of initial weight).	Final weight of skin. (gm.)	Pulp/ Skin weight ratio.	Final condition.
1	127·80	105·05	17·80	21·95	3·79	Skin completely brown, slight fungus growth.
2	124·20	100·67	18·95	21·54	3·68	Pulp watery, no fungus.

Four other fingers from the same row and hand of this bunch were used to investigate the relationship between the transpiration and respiration rates. The methods used in the determination of respiration rate have been described elsewhere (Wardlaw and Leonard, 1939), and the limitations in the control of temperature and humidity within the respiration chamber discussed. Two contrasting humidities were used: approximately saturated air was obtained in one case by the use of a wash-bottle containing a saturated solution of potassium oxalate, and in the other approximately 75 per cent. relative humidity by the use, for the first twenty-four hours, of saturated calcium chloride, thence, up to the climacteric, of a saturated solution of sodium bromide, reverting to saturated calcium chloride when the rate of transpiration had risen to higher values. Duplicate estimations were made at each humidity using a single fruit, and closely comparable curves of respiration and
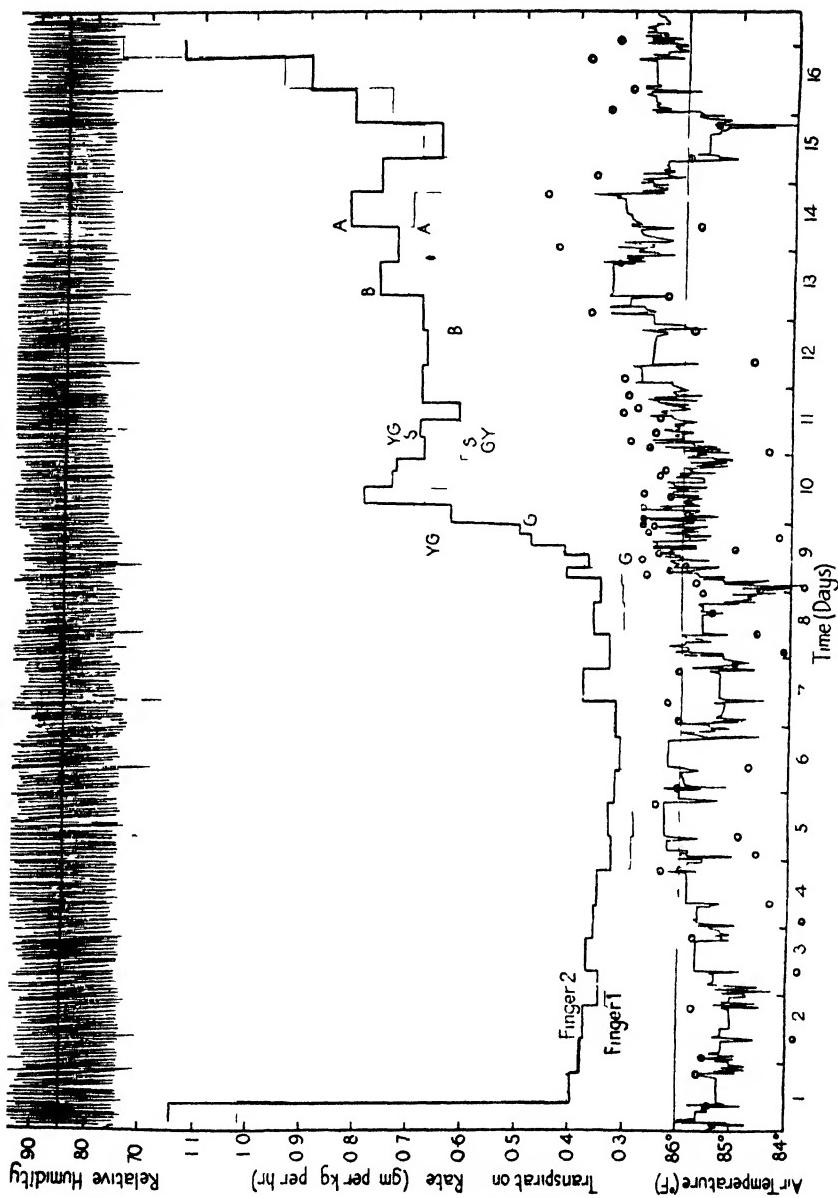
Fig. 10. Rates of transpiration of two fingers from the upper row of the fourth hand of a ten-hand '⅓-full' bunch of Gros Michel bananas (that used for Fig. 9) during ripening at 85°–87° F. Notations for skin colour, &c., as Fig. 5. Temperature records from bimetallic thermograph, together with point observations from mercury-in-glass thermometer. Humidity records from hair hygrograph.

transpiration rates were obtained as between duplicates. Respiration rates are calculated on the basis of the fresh weights of the fruit at the last weighing prior to each respiration period. A record at high and one at low humidity are given in Figs. 11 and 12 respectively, together with air temperatures within the respiration chambers (from mercury-in-glass thermometers) and relative humidities (paper hygrometers). The corresponding observations for the storage-room air are those of Fig. 10; the major ripening changes are indicated. No increase in respiration rate was found as a result of handling the fruit during weighing such as that recorded by Audus (1935) for leaves of cherry laurel.

A discussion of the respiration data has been given in another contribution in this series (Wardlaw and Leonard, 1940). Here attention is directed solely to the relationship in time between the two gas-release phenomena. Additional data are given in Table VIII together with the weight and pulp/skin ratio of a finger from the same hand and row obtained at the start of the experiment.

TABLE VIII

Finger.	Humidity.	Initial weight. (gm.)	Final weight. (gm.)	Loss (% of initial weight).	Final weight of skin. (gm.)	Final pulp/ Skin ratio.	Condition. Skin.	Pulp.
3	High (100%)	125·39	114·44	8·73	30·07	2·81	Completely brown and soft, considerable mycelium.	Watery.
4	Low (75%)	123·67	89·86	26·11	15·10	4·95	Completely brown and wrinkled.	Watery except immediately under skin.
5	—	121·75	—	—	Initial. 46·82	1·60		

Although the experimental conditions could be improved, the change in level of the rate of transpiration is well marked in both fingers, especially that at low humidity; it resembles the records given for banana fingers exposed to the storage-room atmosphere. In both Figs. 11 and 12 the rise in transpiration is seen to coincide with that in respiration, the peak of the respiration climacteric agreeing with the attainment of the new, relatively steady, high rate of transpiration and, in Fig. 12, the final rise in transpiration rate agreeing with that of respiration on the onset of fungal wastage.

The rise in temperature of the air in the respiration chamber during the climacteric will itself be a factor in causing the increase in the respiration rate and, by reducing the relative humidity, in the transpiration rate also. But that such a rise in temperature has a relatively slight effect can be seen by reference to the respiration and transpiration rates before and after the climacteric; with considerable fluctuations in the temperature of the chamber only slight alterations in these rates occur. The fluctuations in the humidity due to the

limitations of the apparatus are also undesirable. It may be noted, in Fig. 12, that the considerable fall in humidity due to the change-over from one salt solution to the other occurred subsequent to the climacteric rise in respiration and transpiration rates. While the actual values are, therefore, open to criticism
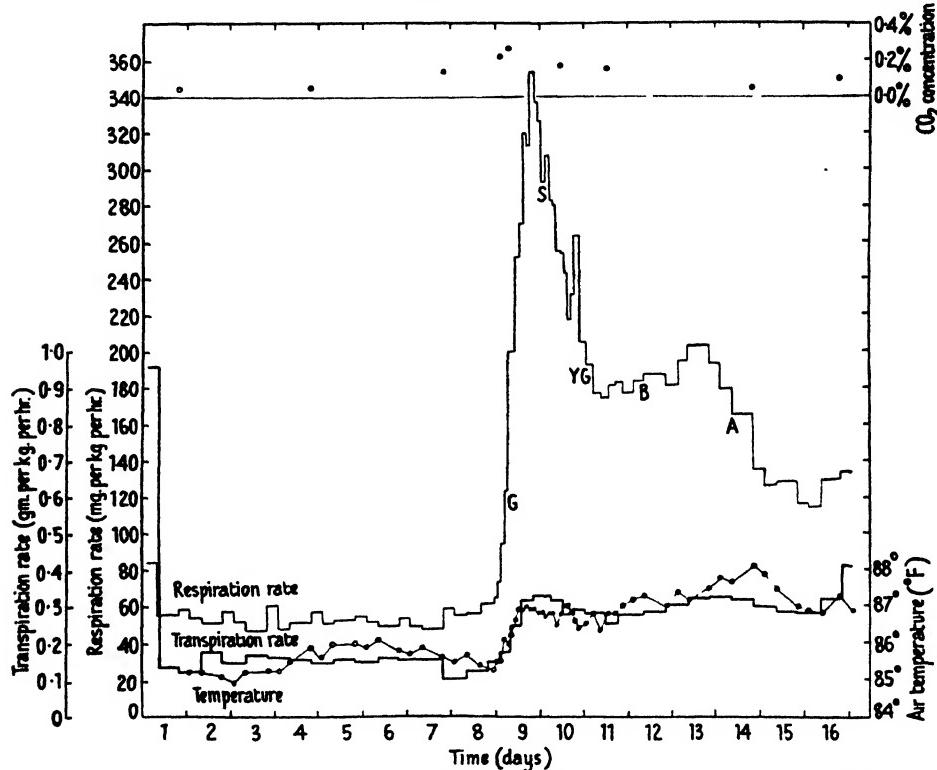
FIG. 11. Rates of transpiration and respiration of a finger from the upper row of the fourth hand of a ten-hand '¾-full' bunch of Gros Michel bananas (companion fruit to those in Fig.10) during ripening at 86°–87° F. in a saturated atmosphere. Temperature of air in respiration chamber by point observations of mercury-in-glass thermometer. Respiration rates by point observations at intervals of carbon-dioxide concentration in air of respiration chamber. Notations for skin colour &c. as Fig. 5.

the time relationship between the rates of respiration and transpiration is well established.

It was calculated that the difference between the water loss from the finger in the saturated atmosphere (Fig. 11) before and after the climacteric rise was approximately the quantity required to increase to saturation the water content of the air at the higher temperature of the chamber during the climacteric.

Olney (1926) using West Indian and Central American bananas after voyages in refrigerated or ventilated holds found the daily loss in weight at 20° C. (68° F.), to be fairly uniform, about 0·4 per cent. of the weight of the fruit per day, of which he states about 25 to 60 per cent. was due to liberation

of carbon dioxide. Hyatt and Turner (1932) note that there is an increase in 'the quantity of "physiological" moisture produced in the later stages of ripening' of Cavendish bananas which necessitates some form of humidity control in commercial ripening rooms, while A. J. M. Smith (1933, p. 148), as a result
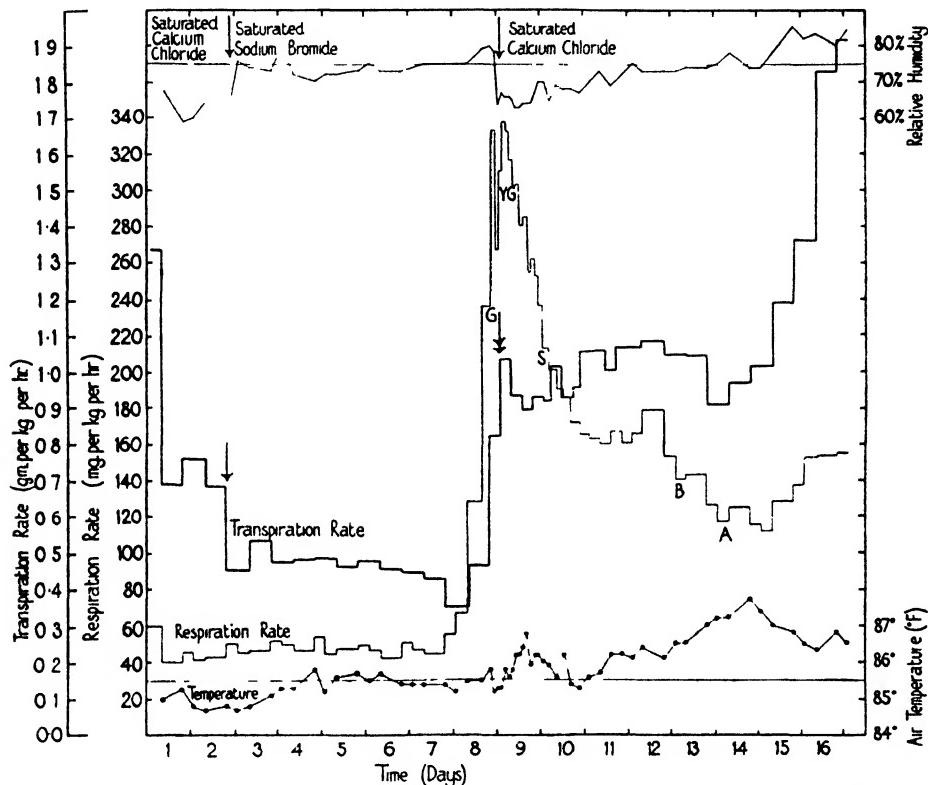
FIG. 12. Rates of transpiration and respiration of a finger from the upper row of the fourth hand of a ten-hand '$\frac{3}{4}$-full' bunch of Gros Michel bananas (companion fruit to those of Figs. 10 and 11) during ripening at 85°–87° F. and about 75 per cent R.H. Temperature of air in respiration chamber by point observations of mercury-in-glass thermometer, relative humidity per cent. by paper hygrometer. Notations for skin colour &c., as Fig. 5. The times at which the material controlling the humidity were changed are indicated by arrows.

of calculations of heat production based on respiration data, suggests that during the climacteric the rate at which bananas lose water will be increased in approximately the same ratio through the incidental lowering of the relative humidity of the air stream; however, he gives no experimental results.

IV. DISCUSSION

The experimental data given here do not provide the basis for a detailed discussion of the water relations of fruit during ripening, but future investigation of the different factors involved demand a brief assessment of those known

to be of importance. They may be divided into external, environmental factors, i.e. temperature, humidity and air movement, and internal factors, such as tissue resistance to the movement of water as vapour or liquid. The environmental conditions of the experiments described, i.e., constant temperature and humidity and rate of air flow, restrict the possible changes to (i) the vapour-pressure gradient between the fruit and air—that of the latter remaining constant—and (ii) the tissue resistance.

(a) The phases of transpiration during ripening.

The changes observed in the transpiration rate may for convenience be divided into four fairly clearly defined stages, some or all of which are shown in the transpiration rates of the four different fruits examined. They are (i) an initial fall in transpiration rate following harvesting, (ii) a relatively steady rate during the green, unripe stage, followed by (iii) a rise and subsequent fall coincident with the onset of ripening, and (iv) a new level (above that of (ii)) which is maintained during ripening; this last may or may not be followed by a final rise with the onset of fungal wastage. The full succession of phases is exemplified in mangoes and bananas; in the tomato only phases (i) and (ii) were observed; while the papaw showed phases (i) and (ii) and a continuous rise in transpiration rate during ripening.

Stage i. The initial fall in transpiration rate[1] following harvesting may be attributed to the setting up of a new gradient of vapour pressure between the epidermis and stomatal cavities and the underlying tissues, to take the place of that existing when the fruit was attached to the parent plant and receiving water. It is noticeable that this initial fall is most marked in bananas the fingers of which are not detached from the bunch until very shortly before the initial weighing, whereas with the other fruits an appreciable interval elapsed between harvesting and weighing.

W. H. Smith (1930), using Bramley's seedling apples in still air at 3° C., found an almost constant rate of loss of fresh weight at 95 per cent. R.H., and a small falling off in rate at 75 per cent. R.H. during storage for six months. Later (1931) he found at 15° C. and 75 per cent. R.H. a decrease of 32 per cent. in the above rate during the first 50 days, but no sharp changes at any time under constant environmental conditions.

Respiration data show, following harvesting, that there is a rapid initial fall in the rate of liberation of carbon dioxide from full grown fruit, accompanied by a decrease in the internal concentration of this gas. The further investigation of this particular aspect of the problem will call for field observations on transpiration, &c., prior to harvesting; these must be deferred. With bananas it is known from biochemical observations also that the period immediately

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[1] It may be noted that the initiation of an abscission layer, which may precede fruit fall, must bring about important changes in the water relationships whilst the fruit is still attached to the plant. The anatomical aspect of this has been the subject of a contribution from this Station (Barnell, 1939).

following harvesting is of very considerable importance in relation to subsequent cold storage (Barnell, 1940).

The initial readjustment of the temperature of the detached fruit to approximately that of the storage room is important in relation to transpiration. A fall in transpiration rate has been found, however, even when the storage-room temperature was higher than that of the air by which the fruit had been surrounded previously. In experiments subsequent to those detailed above 'transition effects' in the transpiration rate similar to those found in the respiration rate have been observed when unripe bananas (fingers or hands) are removed from a lower to a higher temperature, i.e. a rise followed by a fall to a second level above that at the lower temperature.

Stage ii. A relatively steady rate of transpiration, or a slightly declining one, follows the initial fall. It is suggested that this marks the establishment of a fresh equilibrium with a gradient of vapour pressure from saturation somewhere within the fruit to the vapour pressure of the outside air, which may itself be at saturation but at a lower temperature and therefore exerting a lower pressure. The supply of water from the inner tissues by translocation and production as metabolic water reaches a steady value balancing that lost in transpiration. This phase represents the most important period in the storage life of fruit.

Stage iii. The rise in transpiration rate with the climacteric rise in respiration rate is accompanied by a rise in temperature of the tissues. It is evident that altered temperature gradients will be set up from the interior of the fruit to the skin during this rise, accompanied by a redistribution of the vapour-pressure gradient. Preliminary observations on the rise in pulp temperature during the climacteric recorded elsewhere (Wardlaw and Leonard, 1940) show a maximum observed difference in temperature of $1 \cdot 1°$ F. for a finger at an air temperature of $85°$ F.

Stage iv. The post-climacteric phase has been shown in respiration studies (Wardlaw and Leonard, 1936a, 1938, 1939, 1940) to be a period of increasing tissue resistance to the movement of gases. It is marked, in bananas and mangoes, by a period of relatively steady transpiration followed, with the attainment of final senescence, by a rise accompanying the onset of fungal wastage of the skin and outer tissues. The pulp temperature, as ascertained in preliminary experiments on papaws (Wardlaw and Leonard, 1938), remains fairly steady whereas the transpiration rate rises progressively; in bananas (Wardlaw and Leonard, 1939, 1940) the pulp temperature remains relatively steady or may show a gradual decline. A considerable extension of experimental work will be necessary before the relationship between these tissue changes and transpiration can be understood.

It is realized that the three types of transpiration trend found for the four fruits may not include all possible variations, that the division into phases of what is really a continuous process is arbitrary, and that the same fruit under different storage conditions may show different trends. As an illustration of

the last point, bananas stored continuously at 53° F. show only the initial fall in transpiration rate followed by a steady level, although the respiration rate shows a climacteric rise and fall.

(b) *The vapour-pressure gradient.*

Transpiration, as a diffusion phenomenon, must take place along, and be proportionate to, a diffusion gradient of decreasing partial pressure of water vapour. Its investigation therefore calls for an examination of this gradient. In respiration it has been shown that there is a direct relation (though not necessarily a constant one) between the internal concentration and rate of liberation of carbon dioxide (Wardlaw and Leonard, 1936a, 1940), and also that any increase in the external concentration of carbon dioxide is accompanied by an approximately equal rise in the internal concentration of that gas (Wardlaw, 1936). Recently the gradient of vapour pressure involved in transpiration has also been tentatively explored (Curtis, 1936a; Ramsay, Butler, and Sang, 1938; Shaw, 1935; Thut, 1938, 1939). Its investigation demands considerable diversity of methods.

The importance of a knowledge of the temperature of the transpiring body has been stressed by several workers. Brown and Escombe (1905) point out that any rise in temperature of the leaf, no matter how small, will increase the partial pressure of the water vapour of the interspaces of the leaf, a diffusion potential will be produced and water vapour will pass from the leaf into the surrounding air. More recently Curtis (1936) has pointed out that 'a change of only 1° to 2° C. in leaf temperature, may bring about a change in vapour-pressure gradient between the leaf and the atmosphere equivalent to changing the external humidity as much as 5 to 14 per cent. Assuming that at the beginning the leaf is at the same temperature as the air and that the intercellular spaces remain saturated, a rise in temperature of the leaf to 1°C. above the air temperature would be comparable, in its effect on transpiration, to lowering the external humidity by 5·5 to 6·9 per cent. over the temperature range between 40° and 10° C.'

(c) *Tissue resistance.*

The movement of water from the interior of fruits to the ultimate transpiring surfaces will be subject to resistance in both the liquid and gaseous phase. The elucidation of this resistance will require examination of the histology and water content of the tissues; some of the internal factors may be enumerated. The dimensions of (i) the tissue cavities immediately adjacent to the external atmosphere (stomata and lenticels); (ii) the intercellular spaces of the underlying tissues; (iii) the nature, dimensions, and changes in the nature and dimensions of the cell-walls of the epidermis and underlying tissues; (iv) the vapour pressure in the cavities and intercellular spaces of (i) and (ii) and of the adjacent cell-walls; (v) the vapour pressure of the cell contents, solutions, colloidal sols and gels, and the change in these cell contents.

In leaves the functioning of the guard cells of the stomata in the control of transpiration has received considerable attention. In fruits it is uncertain whether there is any considerable mobility of the stomata where these are present in a modified form. The following brief observations on the fruits used in the present experiments are submitted. Tomato fruits possess no stomata: in mangoes the stomata appear to be modified to lenticels: the papaw has a considerable covering of waxy epidermis and the stomata possess a certain degree of mobility. Preliminary observations on the stomata of '$\frac{3}{4}$-full' bananas during ripening have shown that from the time of picking to the 'sprung' condition the stomata remain closed irrespective of the environmental conditions. After the 'sprung' stage there is a tendency for some stomata to open (E. Barnell, unpublished data). Since this condition is subsequent to the climacteric rise it does not appear that a temporary opening of the stomata of bananas is responsible for the increased rates of respiration and transpiration; nevertheless the stomata are not functionless but will open when sections of epidermis are placed in water. Further observations on the role of stomata and lenticels in the gaseous exchanges of fruits are obviously desirable. Apart from any changes in the stomatal aperture the progress of desiccation, especially of the superficial tissues in the absence of any water uptake, would seem to tend towards a reduction rather than an increase in the transpiration rate up to the point when fungal invasion of the skin tissues occurs.

Similar comments on the morphology of skin tissues were put forward in the first contribution of this series (Wardlaw and Leonard, 1936a) with special reference to respiration, and it was further suggested, in regard to the similarity of respiration, transpiration, and surface-bulk curves for fruit of different sizes, that 'with transpiration, where the sub-epidermal tissue is at saturation, the gradient (of vapour pressure) is presumably constant'. A reconsideration of this view now appears necessary.

Even less is known of the effect of the inner tissues on gaseous movement. W. H. Smith (1931) suggests that the rate of loss of water is governed by the resultant of a number of internal factors the precise nature of which is as yet undetermined. In a preliminary investigation of the internal factors he found that apples with the thickest cuticle lost water at the greatest rate, that under the same conditions the evaporation from a free water surface was seventy times that from the apple, and that there was a 50 per cent. increase in the rate of evaporation after death by fungal disease (1932). He later (1938) found that in seven varieties of apple the magnitude of the intercellular volume was in no way related to the size of the cells nor, within a variety, the weight of fruit to the magnitude of the intercellular volume. Apples of varieties with the largest number of cells per unit weight of cortex had the highest, those with the smallest number of cells the lowest, rate of respiration. Markley and Sando (1931) observed that up to 30 per cent. of the total loss in weight of apples took place from the calyx and stem ends.

The continuous loss of water in transpiration from fruit in storage is important in determining the changes in resistance of the tissues to the movement of water and water vapour. There is also the question of the replenishment of the water content by water produced in metabolism. The importance of such metabolic water during the ripening of fruits has been discussed by Babcock (1912). He states that 'nearly all of the changes occurring in the ripening of fruit, except those resulting from direct oxidation are hydrolytic in character and cause liquid water to disappear. The water thus fixed in organic combination may in some cases exceed the metabolic water resulting from oxidation and still leave the ripe fruit far more succulent than the green fruit because more of the organic matter of the ripe fruit is dissolved in the fruit juices.

'It often happens, when fruit is stored under conditions which retard evaporation, that the metabolic water produced by oxidation, is more than sufficient to replace that lost by evaporation and that fixed in organic combination, in which case, the absolute amount, as well as the percentage of water in the ripened fruit, exceeds that present in the green fruit when it was picked.'

Babcock's determinations of the water content of pears, apples, plums, and persimmons showed slight but consistent increases (1 to 2 per cent.) during ripening.

From determinations which showed about the same percentage moisture content in green and ripe strawberries Gerhart (1930) suggests that as a result of the production of metabolic water approximately the same amount of water exists in the fruit over the entire ripening period. Hinton (1934) found very little relation between the rate of loss in weight and the relative water content of the apple, but notes that with Newtons the water content increases during storage. In bananas Gore (1914) has calculated the water formed by respiration and that utilized by the hydrolysis of starch, and has found that except when the banana becomes overripe the water formed in respiration does not equal that used in hydrolysis. Data for mangoes (Cheema, Karmarkar, and Joshi, 1939) show for a large number of varieties little difference between the percentage of water in green and ripe fruit. For the Alphonso variety, however, there was an increasing percentage of water during storage at temperatures between 96° and 60° F. but no alteration at temperatures between 52° and 30° F.

V. Conclusion

The climacteric has been shown to be a period of increased rate of liberation of carbon dioxide, water, and heat. There will be an increased production of metabolic water accompanying the increased CO_2 production derived from the aerobic breakdown of metabolites, which will account in part for the increased supply of water for transpiration.

Essentially the climacteric is a transition phase in which the tissues are

passing from a low level of metabolic activity to a higher one. It is suggested that the exact form of the curves of rates of liberation of gases during this transition may bear no direct relation to the activity of the organ as a whole, since the changes in tissue metabolism may not be a steady progression but a step-by-step succession; whereas, as discussed elsewhere (Wardlaw and Leonard, 1940), gas escape phenomena in transition effects are distorted due to diffusion lag. Such distortion is especially marked where physical effects, e.g. rise in temperature of tissues, occur simultaneously. Until the water content, its state ('free' or 'bound'), its organographic and histological distribution in flesh and skin, and the concentrations and distribution of the metabolites have been further elucidated by biochemical studies (which have been begun), it is not satisfactory to speculate on the relation between transpiration rate and water content. But it is evident that until the last stage of senescence is reached the liberation of water from the surface of the fruit is controlled, at different levels, by various internal factors; in all cases it is considerably less than evaporation from a free water surface.

The further investigation of water losses during ripening will call for exploration of the gradients of vapour pressure, of temperature, and of water content from the surface to the interior of the fruit. Methods are available for obtaining some of these values; their utilization will inevitably provide valuable data on another aspect of the metabolism of fruit in storage for correlation with observations on respiration and biochemical trends.

Closer control of the environmental temperature and humidity than is at present possible at this Station will obviously be required before critical data on changes at the surface and within the fruit tissues can be obtained.

SUMMARY

1. The trends of the transpiration rate of tomatoes, papaws, mangoes, and bananas during ripening at 85° F. have been investigated by determination of rate of total loss in weight.

2. Three types of trend have been found: (i) tomatoes show an initial fall followed by a steady transpiration rate; (ii) papaws an initial fall followed by at first a steady and then a continuously rising rate; (iii) mangoes and bananas show an initial fall followed by a steady rate, and subsequently a trend similar to that of the respiration climacteric.

3. In the papaw the relation in time between the internal concentration of carbon dioxide and oxygen and the transpiration rate has been followed, and that between the respiration rate and transpiration rate in individual banana 'fingers'.

4. The transpiration rates of the individual 'hands' of a bunch of bananas are given, together with the changes in the pulp/skin ratio and a composite curve for the transpiration rate of an intact bunch deduced.

5. The factors which may be responsible for the observed trends of tran-

spiration are briefly discussed in relation to future investigations, emphasis being laid on the effect of changes in temperature of the tissues on the vapour-pressure gradient and on changes in the water content of the tissues.

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The Mycorrhizal Relations of Larch

II. The Role of the Larch Root in the Nutrition of Boletus elegans Schum.

BY

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With Plate III

I. Introduction

IN the previous paper of this series (How, 1940) the writer called attention to the problem presented by the nutrition of a fungus such as *Boletus elegans* which, in pure culture at least, is incapable of using any but the simplest organic compounds; these are present only in traces in the soil and are probably immediately utilized on formation by more rapidly growing soil fungi. The only other nutrient source would appear to be the root with which the fungus is in association. To test this possibility experiments have now been carried out in which excised root-tips of conifer seedlings have been added to pure cultures of the fungus. The present paper records the results, which throw light not only upon the importance of the root as a nutrient source but also on the probable mechanism operating to produce the specificity of *B. elegans* for larch.

II. Experimental Procedure

Conifer seeds were hand picked, scraped clean of wings, and centrifugalized for five minutes in water to wet them thoroughly. After sterilizing in calcium hypochlorite for fifteen minutes, the seeds were washed in sterilized distilled water and germinated in Petri-dishes on damp filter-paper at room temperature (Wilson, 1915). Primary root-tips, varying in length from 2 to 8 mm., were cut off with a sterilized scalpel as required.

Standard culture methods were used throughout, except in experiment 1.

The nutrient medium was as follows: glucose 0·5 per cent., NH_4Cl 0·05 per cent., KH_2PO_4 0·1 per cent., $MgSO_4.7H_2O$ 0·01 per cent., agar-agar (water-washed) 2 per cent. The medium was sterilized by autoclaving at 15 lb. pressure for fifteen minutes. A substrate containing a low concentration of nutrients was used in order to avoid the deleterious effects of high concentrations (How, 1940); preliminary investigations indicated, however, that the concentrations employed were well above the maximum nutritional requirements of the fungus.

Small Petri-dishes (5·5 cm. in diam.) containing 10 c.c. of the medium were inoculated by disc inocula from beerwort agar cultures, the inocula being leached in sterilized distilled water to remove all traces of nutrients and staling products, since it was found that leaching greatly increased the growth rate (Pl. III, Fig. 1, cf. A and C with B and D). In cultures containing root-tips one inoculum was placed in each dish in close contact with an excised root.

The projection method previously described (How, 1940) was used to measure the areas of the cultures in all but experiment 1; the values thus obtained were analysed statistically, using the 't' test of Fisher (1930, section 24.1). It should be realized that the measurement of surface area takes no account of the marked differences in density of the cultures resulting from stimulation; however, any method involving the weighing of mycelium is ruled out by the necessity of using solid media and the presence in many cultures of root-tips of unknown weight.

III. Growth-promoting Qualities of Larch Roots

Experiment 1. This was a preliminary experiment in which only three dishes were used in each set.

    Series I. Plain agar medium.
        A. Unleached inocula
        B. Leached   ,,
        C. Unleached   ,,   +root-tip of European larch
        D. Leached   ,, + ,,   ,,   ,,   ,,
    Series II. A similar set in which the nutrient agar was used.

In series I germination of the inocula was obtained but no further growth. Evidently with no other external supply of food material the fungus was unable to utilize whatever nutrients were contained in the root, probably because of its inability to enter the root before the traces of nutrients available to it were exhausted. In series II, however, the presence of a root-tip gave a marked stimulus to growth, the average diameters of the cultures being nearly doubled (Pl. III, Fig. 1). It is impossible that in these cultures the increased growth-rate was due to additional carbon or nitrogen sources available to the fungus on entry into the root, since the nutrients provided in the agar were known to be more than sufficient for growth. It is concluded, therefore, that the stimulant is in the nature of a growth-promoting substance rather than a nutrient. If this is so, it becomes of interest to discover whether the growth

promoter is specific to European larch. The following experiments indicate that it may be.

Experiment 2. Single excised root-tips of European larch, Japanese larch, and Scots pine seedlings were tested on inocula growing on the standard medium; there were six cultures in each set. Table I shows clearly that a stimulus to growth is produced by roots of both European and Japanese larch, but that only a slight stimulus occurs with Scots pine. Statistical analysis of the areas of the cultures after thirteen days' growth indicates that while the mean of the Scots pine cultures differs only just significantly from that of the controls which contained no roots, those of the European and Japanese larch cultures show a highly significant difference. (Table II.)

TABLE I

Areas (sq. cm.) of Cultures 13 Days after Inoculation

Control Fungus alone.	Fungus + European larch root.	Fungus + Japanese larch root.	Fungus + Scots pine root.
5·22	7·14	7·58	5·83
4·53	6·72	7·33	5·06
4·22	6·55	6·64	5·00
3·89	6·05	5·94	5·00
3·64	5·44	5·86	4·94
3·05	4·78	4·89	4·11
Mean[1] 4·09 ± 0·75	6·11 ± 0·87	6·37 ± 1·0	4·99 ± 0·54

TABLE II

Value of 't'

Treatments compared.

Control and European larch root . .	4·33 (P = 0·01)
,, ,, Japanese ,, ,, . .	4·45 (P = 0·01)
,, ,, Scots pine root . .	2·37 (P - 0·05)
European larch and Scots pine roots .	2·66 (P 0·05)

Experiment 3. The previous experiment was repeated using European larch and Scots pine roots only. A marked stimulus was again obtained with European larch, but only a negligible stimulus with Scots pine (see Tables III and IV). Statistically the mean area of the European larch cultures after fourteen days showed a highly significant difference from that of the controls, while the mean of the Scots pine cultures was not significantly different. However, the Scots pine cultures showed considerable variation among themselves; three of the cultures reached values as high as those of the poorest European larch cultures, giving an impression of definite stimulation.

Experiment 4. This was designed to discover whether the observed results

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[1] The standard error of the mean is shown in this and subsequent tables.

were due to differences in the quality of the growth promoter in Larch and Pine roots or only in the quantity. Four series of cultures were set up containing no roots, one European larch root, four European larch roots, and four Scots pine roots, respectively. It is clear from Table V that the increase in

TABLE III

Areas (sq. cm.) of Cultures 14 Days after Inoculation

Control	Fungus + European	Fungus + Scots pine
Fungus alone.	larch root.	root.
4·33	6·33	5·5
3·97	6·05	4·92
3·64	5·72	4·69
3·55	5·61	3·55
3·22	5·22	3·50
3·17	5·08	3·39
3·06	5·00	3·22
2·94	4·83	3·08
2·44	4·77	2·89
2·22	4·44	2·72
Mean 3·25±0·65	5·3±0·61	3·75±0·95

TABLE IV

Values of 't'

Treatments compared.

Control and European larch root . .	7·32 (P = 0·01)
„ „ Scots pine root . .	1·38 (P 0·2)
European larch and Scots pine roots .	4·38 (P = 0·01)

TABLE V

Areas (sq. cm.) of Cultures 14 Days after Inoculation

Control	Fungus + one root of	Fungus + four roots	Fungus + four roots
Fungus alone.	larch.	of larch.	of pine.
3·53	4·28	5·22	3·00
3·28	3·67	4·72	2·72
3·05	3·39	3·61	2·72
2·67	2·89	3·47	2·61
2·22	2·39	3·39	2·22
Mean 2·95±0·52	3·32±0·73	4·08±0·83	2·65 ±0·28

the number of Larch roots results in increased growth of the fungus, but a similar increase in the number of pine roots does not increase the growth but tends rather to depress it. Unfortunately it was only possible to set up a small number of cultures in each series, thus considerably reducing the likelihood that significant differences would be found between the mean values. However, the mean of the areas of the 'four-root' Larch cultures was significantly greater than that of the controls, although the mean of the 'one-root' cultures was not; moreover, the difference between the 'four-root' larch cultures and the 'four-root' pine cultures was highly significant (see Table VI).

TABLE VI

Values of 't'

Treatments compared.

Control and one-root larch . . .	0·92 (P = 0·4)
„ „ four-root „ . . .	2·58 (P = 0·05)
„ „ „ pine . . .	0·54 (P = 0·7)
Four-root larch and four-root pine . .	3·63 (P = 0·01)

It is thus evident that the differences in growth cannot be attributed to differing amounts of one growth substance but are due to a difference in quality in the substances offered to the fungus by the root. It is therefore concluded that a substance which promotes the growth of *B. elegans* is present in the primary roots of European and Japanese larch but not in those of Scots pine. There may be other growth-promoters which are occasionally present in sufficient quantity in pine roots to stimulate the growth of the fungus as was observed in experiments 2 and 3, but it is clear that no sign of the existence of such substances appeared in experiment 4, where their effect might be expected to be four times as strong. This discrepancy points, no doubt, to the reaction of the fungus being due to the influence of a number of substances in the root, some stimulating and some inhibiting; but the technique so far employed is too crude to detect this with certainty. The evidence at present available only warrants the conclusion that a growth-promoting complex is present in larch roots but not in Scots pine roots. The term growth-promoter, therefore, is used in this paper merely to cover the phenomena recorded in the experiments, and with no suggestion that one is necessarily concerned with a single substance.

IV. NATURE OF THE GROWTH-PROMOTER

Experiment 5. Five excised roots were crushed with a sterilized glass rod under aseptic conditions, and each was left overnight in 60 c.c. of sterilized distilled water; five other roots were crushed similarly but were not washed. The growth rates obtained from cultures to which these treated roots had been added were compared with others containing one untreated root and no root (Tables VII and VIII).

TABLE VII

Areas (sq. cm.) of Cultures 19 Days after Inoculation

	Control	Fungus +	Fungus +	Fungus +
	Fungus alone.	Uncrushed root.	Crushed root.	Crushed and washed root.
	3·39	8·28	5·39	4·5
	3·00	5·33	5·22	3·61
	2·58	4·78	4·94	3·22
	2·39	4·72	4·33	2·72
	1·69	3·83	3·55	2·5
Mean	2·61±0·64	5·39±1·7	4·69±0·75	3·31±0·79

TABLE VIII

Values of 't'

Treatments compared.

Crushed root and uncrushed root	.	.	0·84 (P = 0·5)
,, ,, ,, crushed and washed root			2·82 (P = 0·05)
Control ,, ,, ,, ,,			1·53 (P = 0·2)

Experiment 6. This was a repetition of the previous experiment with four cultures in each series. The results are recorded in Tables IX and X.

TABLE IX

Areas (sq. cm.) of Cultures 14 Days after Inoculation

	Control Fungus alone.	Fungus + Uncrushed root.	Fungus + Crushed root.	Fungus + Crushed and washed root.
	4·33	6·33	6·17	4·44
	3·55	5·61	6·03	4·28
	3·06	5·00	4·67	3·61
	2·22	4·44	4·61	2·66
Mean	3·29±0·88	5·34±0·94	5·37±0·84	3·75±0·81

TABLE X

Values of 't'

Treatments compared.

Crushed root and uncrushed root	.	.	0·05 (P	0·9)
,, ,, ,, crushed and washed root			2·77 (P	0·05)
Control ,, ,, ,, ,,			1·74 (P	0·2)

In both experiments the mean areas of the crushed root cultures do not differ significantly from those with untreated roots; but crushing followed by washing in water results in the complete loss of the power to stimulate growth. Microscopic examination of the roots after washing in water shows that many of the cells remain intact, so that the washing probably removed only the water-soluble substances and not the entire protoplasm.

Experiment 7. Since a number of growth-promoters are known to be soluble in chloroform it was decided to test whether any part of the growth-promoting complex in larch roots could be removed with liquid chloroform. Some excised roots were subjected to chloroform vapour for twenty-four hours and others to liquid chloroform. After the evaporation of the chloroform under aseptic conditions the roots were used in the standard way. From Table XI it is seen that treatment with chloroform vapour does not affect the growth-promoting power of the root, but that treatment with liquid chloroform partially removes it. This is only partial, however, since the mean areas of root cultures treated with liquid chloroform differs from that of the controls

highly significantly, but from that of the untreated root cultures only significantly (Table XII). It can therefore be concluded that treatment with liquid chloroform only slightly impairs the effect of the growth-promoter.

TABLE XI

Areas (sq. cm.) of Cultures 19 Days after Inoculation

	Control Fungus alone.	Fungus + untreated root	Fungus + chloroform vapour root.	Fungus + liquid chloroform root.
	3·39	8·28	6·17	5·33
	3·05	5·39	6·06	4·39
	2·78	5·33	5·61	3·82
	2·58	4·78	5·33	3·72
	2·44	4·72	5·11	3·5
	2·39	4·39	4·5	3·5
	1·69	3·83	4·17	3·17
Mean	2·62±0·54	5·25±1·4	5·28±0·75	3·92±0·64

TABLE XII

Values of 't'

Treatments compared.

Untreated root and chloroform vapour root				0·05	(P = 0·9)
„ „ „	„	liquid	„	2·28	(P 0·05)
Control „	„	„	„	4·11	(P 0·01)

Experiment 8. The previous experiment had suggested that complete extraction of the growth-promoter might only be possible if washing in liquid chloroform or absolute alcohol was combined with leaching in water. Accordingly sets of roots were subjected to one of three treatments, either chloroform vapour to kill without extraction, or liquid chloroform or absolute alcohol to extract the fractions soluble in these; all roots were then washed for twenty-four hours in sterilized distilled water before adding to the cultures. When the mean areas of the cultures are compared after fourteen days with the mean area of the controls it is seen that they do not differ significantly from the latter (see Tables XIII and XIV); it is therefore evident that the chief constituents of the growth-promoter can be removed by washing dead roots in water, as well as by crushing and washing. It will also be observed that the results of this experiment do not confirm the suggestion that either liquid chloroform or absolute alcohol remove a further part of the growth-promoter, since the roots treated with liquid chloroform before washing in water give as good growth as those treated with the vapour, in fact slightly better. It should also be noted that unsuccessful attempts were made to demonstrate the exudation of a water-soluble growth-promoter from the roots of larch seedlings grown in culture. Further attempts would have to be made, however,

before this evidence could be used to disprove the solubility of the growth-promoter in water.

TABLE XIII

Areas (sq. cm.) of Cultures 14 Days after Inoculation
(All roots washed in water after treatment)

Control Fungus alone.	Fungus + chloroform vapour root.	Fungus + chloroform liquid root.	Fungus + absolute alcohol root.	
3·53	3·55	3·58	3·39	
3·28	2·83	3·5	3·00	
2·67	2·69	2·83	2·72	
2·22	1·78	2·72	2·17	
Mean 2·92±0·59	2·71±0·73	3·16±0·44	2·82±0·51	

TABLE XIV

Values of 't'

Treatments compared.

Control and chloroform vapour root   .   0·45 (P = 0·7)
      „   „   „   liquid   „   .   0·65 (P = 0·6)
      „   „   absolute alcohol   „   .   0·26 (P = 0·9)

V. MICROSCOPIC EXAMINATION OF THE ROOT-FUNGUS CULTURES

Living cultures of both infected and uninfected root-tips were examined. For the first two days the root-tips continued to grow, as shown by the development of geotropic curvature and for about ten days the root cells appeared to be living, there being no sign of plasmolysis; after this period many of the cells showed signs of disorganization. There were no indications that infection by *B. elegans* either hastened or delayed the death of the root cells, except where these were invaded by mycelium.

It was noted that hyphal strands were particularly numerous in root cultures while they were never present in the controls; this confirms Melin's observation (1922) of a marked stimulus to the production of hyphal strands in mycorrhizal synthesis experiments with larch seedlings.

For detailed investigation of the penetration of the root by the fungus the cultures were fixed in Doak's fixative: 50 per cent. alcohol 90 c.c., 40 per cent. formalin 8·5 c.c., glacial acetic acid 1·5 c.c.

When hand sections of infected larch roots were examined it was found that the fungal mycelium rapidly formed a thick mantle round the root; comparison showed, however, that the hyphae were more loosely woven than in normal larch mycorrhiza formed by *B. elegans* in the soil. Scattered irregularly through the mantle were isolated cells of the cortical filaments and outer cortex (see Melin, 1922, for the structure of the cortex in larch roots). Directly within the mantle the cells were often filled with a dense hyphal

tissue and in some cases the cells so filled had burst, fragments of their cell walls lying embedded in the mantle. It was not possible to determine how far penetration into the root could go, since staling of the cultures intervened even before the inner cortical cells were reached. Pressure from the growing hyphae would appear to be the main cause of the disintegration of the root; for so many isolated and empty cortical cells remain in the mantle that penetration by means of mechanical weaknesses in the walls seems more probable than absorption of the cell membranes (Pl. III, Fig. 2).

A structure similar to the above is exhibited by Scots pine roots infected by *B. elegans*, but penetration is less deep; only a few scattered cells of the outer cortex become absorbed into the mantle, and entry of cells by hyphae is rare. Scots pine roots appear to resist the fungus better than larch roots, possibly because the outer cortical cells in Scots pine have somewhat rigid walls. It was interesting to observe that although larch roots infected with *Penicillium* sp. showed less disintegration than those infected with *B. elegans*, the typical appearance of the root was essentially similar; there was a mantle enclosing isolated cortical cells and the broken walls of others. It seems probable, therefore, that the phenomena observed in all the excised roots infected with fungi is the result mainly of pressure produced by growing mycelium on a relatively soft tissue.

In conclusion, there is no indication that the morphology of excised larch roots infected by *B. elegans* corresponds in any way to that found in true mycorrhiza formation; it is clearly a case of quasi-parasitic invasion by the fungus. This being so, it is noteworthy that the description and the photomicrograph of roots of larch seedlings grown in pure culture and infected with *B. elegans* as given by Melin (1922) are very similar to those recorded in the present experiments; there is, for example, no tannin layer present in either case. It is probable that his seedlings were parasitized by the fungus and that no true mycorrhiza formation took place.

VI. DISCUSSION

It has been established in the present paper that excised roots of Japanese and European larch seedlings are capable of stimulating the growth of *B. elegans*; and that no such power of stimulation is possessed by roots of Scots pine. This at once suggests an indication of the mechanism which brings about the specificity of *B. elegans* for larch. Thus in the soil the growth-promoter stimulates the fungus to greater growth in the immediate vicinity of larch roots and mycorrhiza formation results. Without such increased growth activity penetration is presumably very difficult; so that mycorrhiza formation does not occur with roots, such as Scots pine, which possess no growth-promoter. This hypothesis is dependent, however, on the future demonstration that the growth-promoter normally exudes from the root; so far there is no evidence for this, though the fact that the growth-promoter is soluble in water renders it possible. The further consideration must not be

overlooked that the entry of the mycelium into the cells in the root-fungus cultures may render substances available to the fungus which are not available in the natural mycorrhizal relationship. If the hypothesis were to be rejected on this ground, an explanation would still be required for the remarkable parallel between the presence of a growth-promoter in the roots of larch, the only genus known to be capable of forming mycorrhiza with *B. elegans*, and the absence of such a substance from the roots of Scots pine in which mycorrhiza formation by *B. elegans* is unknown. Therefore, until definite evidence is forthcoming to the contrary, it may be concluded that one factor bringing about the specificity of *B. elegans* for larch is the presence in the roots of a growth-promoter. If this is so, then it follows that, whatever benefits may or may not accrue to the tree from the mycorrhizal association, the fungus at least can obtain growth-promoting substances.

It remains to consider the nature of the growth-promoter. That this will eventually prove to be a complex of substances rather than a single substance has already been suggested in section III. Apart from the fact that the main constituents of the growth-promoter are soluble in water, the present investigation provides no further information. It is of interest to note that Melin (1922) recorded the stimulation of the growth of *B. elegans* immediately contact had been established with the hypocotyl of larch, spruce, and pine seedlings; but he gave no suggestion as to the cause of the stimulation. There are, however, in the literature two further references to substances capable of stimulating the growth of various species of Boletus known to be mycorrhiza formers. Melin (1925) showed that exudations from pine and spruce seeds had a growth-promoting power; it was suggested that this was due to the presence of phosphatides which Hansteen-Cranner had previously reported to be exuded by coniferous seeds and roots (see footnote, Melin, 1925). Steward (1928) failed to find any evidence for the diffusion of phosphatides from living plant cells as Hansteen-Cranner had claimed, so that the nature of the growth-promoter found by Melin is uncertain. Later, Melin and Lindberg (1939) showed that *B. elegans* is stimulated by yeast extract and vitamin B_1; it is probable that traces of vitamin B_1 are present in larch roots, from analogy with excised tomato roots (Bonner and Greene, 1938), and stimulation might therefore be expected from the addition of such roots to fungal cultures. But neither the stimulator found by Melin in conifer seeds nor vitamin B_1 can possibly be responsible for the phenomenon recorded in this paper, since these stimulators are not specific to larch but occur in many other plant species. Any clue to the identity of the growth-promoter is therefore dependent on its extraction from larch roots and its subsequent investigation.

The bearing of these experiments on the wider issues of the mycorrhizal problem must be mentioned. Melin held that the so-called virulence of the fungus was largely responsible for the type of association which developed between any two partners in a mycorrhizal relationship, strongly virulent fungi became parasitic, less virulent ones formed well-balanced mycorrhizas.

The present experiments serve to emphasize how important a factor is the physiological condition of the host, since weakness of the root due to severance from the parent plant results in the extreme case of unbalanced association described in this paper.

VII. SUMMARY

The effects resulting from the addition of excised primary roots of conifer seedlings to cultures of *B. elegans* have established that a water-soluble substance capable of stimulating the growth of the fungus is present in the roots of European and Japanese larch, but not in those of Scots pine. During the course of the experiments the roots became subject to a quasi-parasitic attack by the fungus, which penetrated by means of the mechanical pressure exerted by the growing mycelium.

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EXPLANATION OF PLATE III

Illustrating Miss J. E. How's paper on 'The Mycorrhizal Relations of Larch. II. The Role of the Larch Root in the Nutrition of *Boletus elegans* Schum.'

Fig. 1. Cultures of *B. elegans* from experiment 1; 13 days old ($\times \frac{1}{4}$ approx.) A. unleached inoculum, no root; B. leached inoculum, no root; C. unleached inoculum + root-tip of European larch; D. leached inoculum + root-tip of European larch.

Fig. 2. Transverse section of an excised primary root of European larch, 15 days after inoculation with *B. elegans*. *a*, mantle of loosely woven hyphae; *b*, group of cortical cells filled with mycelium; *c*, outer empty cortical cells separated from the root by the mantle. ($\times 75$.)

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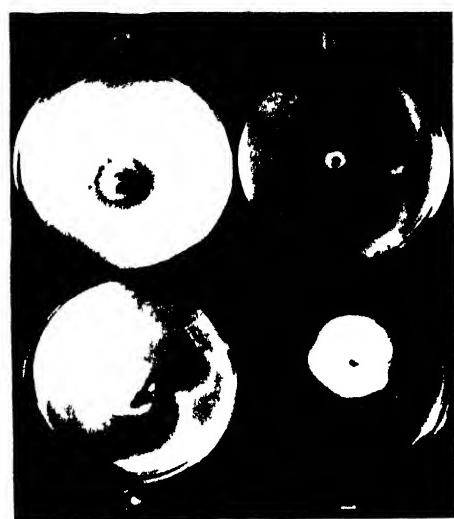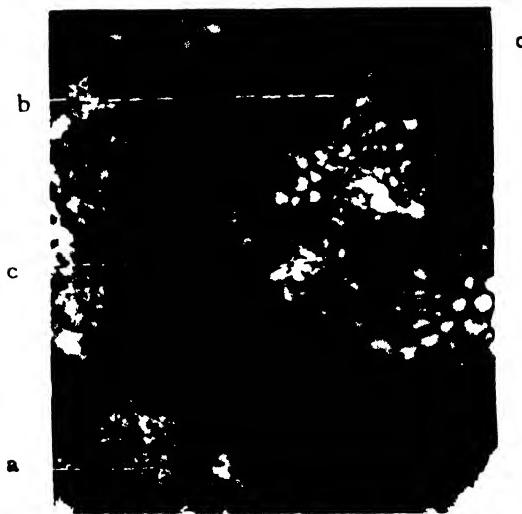
HOW — MYCORRHIZAL RELATIONS OF LARCH.

The Effect of Vitamin B₁ and Nicotinic Acid upon the Growth and Yield of Spring Oats and Tomatoes in Sand Culture

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TO obtain information concerning the factors controlling the growth of plant tissues, physiologists have used small isolated portions of various plant organs and have endeavoured to keep them alive and growing in synthetic media. For instance, root-tips excised from the rest of the plant, and pea embryos shorn of their cotyledons have formed the experimental material of various workers. Under normal growing conditions the growing root-tip receives its nutriment and other substances from the rest of the plant, and the embryo from the store in the cotyledons; when the natural sources of supply are removed it is possible to substitute artificial ones and thus to obtain data upon the likely constitution of the natural materials.

Thus, Kogl and Haagen-Smit (1936), using excised pea embryos, showed that vitamin B₁ had a marked stimulating action upon both root and shoot. Using similar material on an agar medium containing mineral salts and 4 per cent. sucrose, Bonner and Axtman (1937) found that pantothenic acid, vitamin B₁, vitamin C, and folliculin, all increased the rate of growth. When two or more were used together the effect was greater than with one alone, but not equal to their sum. Bonner and Addicott (1937) demonstrated that, for the cultivation of pea roots on an inorganic medium to which 4 per cent. sucrose was added, yeast extract was necessary. The yeast extract could be partially replaced by vitamin B₁ and wholly replaced by vitamin B₁ and a number of amino-acids.

Bonner (1937) has also shown that active growth of excised pea root-tips in a pure synthetic medium could be maintained only by the addition of 0.2γ per c.c. of crystalline vitamin B₁. Even 0.002γ per c.c. produced marked stimulation.

Robbins and White (1937) using excised corn root-tips report that the diffusate from germinating maize grains was beneficial to their growth, and Robbins and Bartley (1937) have found that excised tomato roots would grow in a nutrient solution of mineral salts and pure sucrose with dried brewer's yeast, vitamin B₁, or 4-methyl-5-β-hydroxyethylthiazole added. On the

other hand, White (1937) confirmed that vitamin B_1 was an important factor in the nutrition of excised tomato roots, but that even when supplemented with standard salts, accessory salts, sugar, and nine amino-acids, it was not as effective as was the yeast fraction soluble in absolute ethyl alcohol.

Went, Bonner, and Warner (1938) have obtained important results on the effects of vitamin B_1 and the rooting of cuttings. Aetiolated pea stems were treated basally for 20 hours with indolylacetic acid (20–200 mg./litre) and were then transferred to bottles containing 5 c.c. 2 per cent. sucrose. At different times after the first treatment, vitamin B_1 in a wide range of concentrations was added to the sucrose solutions for different periods. Concentrations of 1 mg./litre or lower applied 5–9 days after the first treatment gave the best response, i.e. several hundred per cent. more roots than in controls not treated with vitamin B_1. Without the first treatment the vitamin was without influence on rooting. Similar results were obtained for lemon cuttings.

That excised pea roots can synthesize vitamin B_1 as determined by the Phytophthora bioassay from a mixture of pyrimidine and thiazole derivatives has been proved by Bonner and Buchman (1938). The formula assigned to vitamin B_1 (as its hydrochloride) is as follows:

$$
\begin{array}{ccccc}
 & N = C.NH_2 \ HCl & & CH_2 & \\
 & & & | & \\
CH_3.C & C \ \cdots\cdots CH_2 \ \cdots\cdots N & C = C.CH_2.CH_2OH & \\
 & & & \\
N - CH & & Cl \ \ CH - S & \\
 & \uparrow & & \uparrow \\
 & \text{Pyrimidine} & & \text{Thiazole} \\
 & \text{nucleus} & & \text{nucleus}
\end{array}
$$
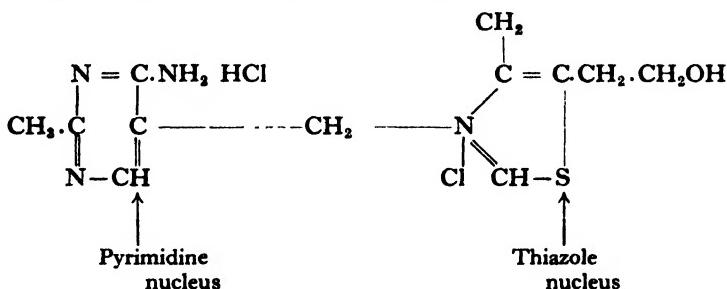
An appreciation of this makes Bonner and Buchman's findings clearer. The reaction occurs *in vivo* under conditions preventing the reaction *in vitro* indicating that a special enzyme thiaminase takes part. It is suggested that a different enzyme system thiazolase is able to effect closure of the thiazole ring from suitable acyclic compounds. In this connexion Bonner's (1938a) results indicate that excised tips of pea roots contain considerable reserves of vitamin B_1 and that in the synthesis mentioned above substituted thiazole derivatives can replace thiazole in such mixtures provided their derivatives possess a hydroxyl group or other group metabolizable to hydroxyl. As pyrimidine constituents, roots can utilize 6-amino-2:5-dimethyl pyrimidines substituted in the 5-methyl position by groups which permit formation of quaternary salts. The 6-amino group cannot be replaced by hydroxyl. The vitamin B_1 content of pea plants (Bonner and Greene, 1938) increases in the light but not in the dark, and root-tips of plants whose leaves are in the light contain more vitamin B_1 than if the leaves are in the dark. It is therefore considered that vitamin B_1 is produced in the leaves in the light and transported

to the root-tip. Also when vitamin B_1 is applied to the roots of plants grown in the dark, both shoot and root growth are increased. The addition of vitamin B_1 (1·0, 0·1, and 0·01 mg./litre of nutrient solution) increased the shoot growth of species normally slow-growing. There was no response with fast-growing annuals. These authors have suggested that the beneficial effects of various manures may be due to their vitamin B_1 content.

From the above results some laboratory evidence has come to indicate the importance of nicotinic acid (pyridine 3-carboxylic acid) for the growth of isolated pea embryos and roots (Addicott and Bonner, 1939; Bonner, 1938). It has been shown that nicotinic acid and vitamin B_1 are equally essential for the continued growth of excised pea roots. For shoot growth, vitamin B_1 and nicotinic acid have caused larger increases in growth than did either alone. Robbins and Schmidt (1938) also found that vitamin B_1 was very effective in small amounts in maintaining growth of excised tomato roots.

Confirming the earlier work with excised pea roots, Addicott and Devirian (1939) state that these may be grown for an indefinite number of weekly transfers in a solution containing only calcium nitrate, magnesium sulphate, potassium nitrate, potassium chloride, dihydrogen potassium phosphate, ferric tartrate, sucrose, vitamin B_1, and nicotinic acid. Other elements, pea ash and twenty amino-acids singly or in combination, could not sustain growth indefinitely in the solution in the absence of nicotinic acid. Isolated roots of pea, radish, flax, and tomato were cultivated by Bonner and Devirian (1939) on a similar medium, and it was found that additions of adenine, pantothenic acid, vitamins B_2, B_6, E, and K did not increase the growth-rate of radish or pea roots. Additions of β-alanine, ascorbic acid, and thalin caused no increase in the case of pea roots, and asparagine, glutamic acid, glycine, isoleucine, leucine, tryptophane, and valine gave no result in the case of radish. For flax vitamin B_1 was necessary, but nicotinic acid and vitamin B_6 were not needed. Both vitamins B_1 and B_6 were necessary for tomato and the addition of nicotinic acid increased the growth-rate. Addicott (1939) has concluded that for pea roots the action of vitamin B_1 as a growth hormone is through an effect on the meristematic activity rather than on cell elongation which is a primary effect of the auxins. Cell elongation, differentiation, and maturation proceeded normally in the roots to which vitamin B_1 was not supplied as far as could be observed, even though meristematic activity was greatly reduced. In this connexion Addicott and Bonner (1939) have suggested that excised pea roots grown *in vitro* under sterile conditions in a complete medium including vitamin B_1 and nicotinic acid may be able to synthesize auxin. Bonner and Greene (1939) have obtained confirmation of their earlier findings that the addition of vitamin B_1 to cultures of certain plants increased the dry-matter production. Plants not responding contained more vitamin B_1 in their leaves than plants which did. It was suggested that the amount of vitamin B_1 synthesized by the leaves of a given species regulates its response to additions of vitamin B_1 and that leaf content may be used to

diagnose whether or not a response is likely. The leaves of plants to which vitamin B_1 was applied contained finally more vitamin B_1 than the controls.

During 1939 Robbins has summarized his views on vitamin B_1 and plant growth and in conjunction with Schmidt (1939, 1939a) has produced further data on this subject. These workers have shown that the greater efficiency of brown sugar than that of pure sucrose in promoting growth of excised tomato roots is not due to its ash constituents. Additions of nicotinic acid, nicotin-amide, or amino-acids to a sucrose-minerals-thiamin medium did not improve growth, but vitamin B_6 produced beneficial effects. Vitamin B_6 also improved growth in a sugar-minerals-thiamin medium in which the proportion of brown sugar was restricted.

In an important recent contribution West (1939) has reported the excretion of significant amounts of thiamin and biotin from the young roots of higher plants, as a normal occurrence even under sterile conditions. 'Bison' and 'Novelty' flax seedlings excreted 0·23–0·64 mg. of thiamin during 1–2 weeks and 0·06–0·20 mg. biotin during 1–3 weeks. Whilst West uses his data to account for certain differences characteristic of the bacterial flora of the rhizospheres, it may be very important from many other aspects if the excretion of vitamin B_1 from roots can be shown to be a normal feature of many green plants.

The two experiments reported in this paper were begun early in 1939 before many of the papers mentioned above had appeared, for it has seemed very desirable to determine the effect of vitamin B_1 and nicotinic acid applied to the growing medium upon the growth of plants, especially those crops of economic importance. Tomatoes and spring oats were chosen and these have been grown in a sand medium, fed with an inorganic nutrient solution, to which the differential treatments were applied. Full details are given for each experiment.

Experiment I: Spring Oats

Experimental.

A pot-culture experiment was set up consisting of seventy-two pots, including twelve treatments arranged in six random blocks. Treatments were as follows:

1. Soil control (1¼ cwt./acre nitrochalk in May).
2. ,, ,, (,, ,, ,,).
3. Sand control (nutrients only).
4. ,, ,, (,, ,,).
5. Sand nutrients + vitamin B_1 0·3 mg./pot weekly.
6. ,, ,, + ,, ,, 3·0 ,, ,,
7. ,, ,, + nicotinic acid 0·3 mg./pot weekly.
8. ,, ,, + ,, ,, 0·3 ,, ,,
9. ,, ,, + vitamin B_1 0·3 mg./pot + nicotinic acid 0·3 mg./pot weekly.
10. ,, ,, + ,, ,, 0·3 ,, + ,, ,, 3·0 ,, ,,
11. ,, ,, + ,, ,, 3·0 ,, + ,, ,, 0·3 ,, ,,
12. ,, ,, + ,, ,, 3·0 ,, + ,, ,, 3·0 ,, ,,

All pots were of glazed porcelain (10 in. × 10 in.) having a 3-in. layer of ¼ in. pea-gravel in the bottom, and an earthenware thumb-pot was inserted centrally in the surface after filling. Soil pots were filled with Berkshire loam, and sand pots with 25 lb. of a mixture of 96·5 per cent. Garsides graded 2L silver sand, 3·0 per cent. prepared calcium bentonite and 0·5 per cent. calcium carbonate. The sand pots were thoroughly leached individually after filling, until the chlorine content of the effluent was steady at 0·06 mg. Cl. per ml. The tubules of all pots were covered with a circlet of fine muslin to ensure adequate drainage. The pots were sown March 11 with Garton's White Victory spring oats (dressed with Agrosan G) at nine seeds per pot, but were thinned out after germination to four seeds per pot (April 17).

Application of nutrients to sand cultures.

The following concentrates were prepared:

Concentration No. 1.			Concentration No. 2.		
$NH_4H_2PO_4$	.	. 161·8 gm.	$NH_4H_2PO_4$	.	. 161·8 gm.
KNO_3	. .	. 858·4 ,,	KNO_3	. .	. 469·4 ,,
$(NH_4)_2 SO_4$	.	. 70·9 ,,	$(NH_4)_2 SO_4$	.	. 70·9 ,,
NH_4NO_3	.	. 131·4 ,,	KCl	. .	. 286·8 ,,
dissolved in 5 galls. water.			dissolved in 5 galls. water.		

The final nutrient solution was prepared from these by dilution thus:

> 517 ml. concentrate.
> 4·5 ml. minor elements concentrate (5 × Normal).
> Water to 1 gallon. (See Appendix II.)

Applications of the above, varying from 100 to 200 ml. of either No. 1 or No. 2 were made at approximately weekly intervals from April 3 to August 10. In general, No. 2 was used in the early stages and No. 1 towards maturity. Between applications the pots received the normal rainfall, tap-water being added as necessary.

Application of nicotinic acid and vitamin B_1.

The following concentrates were prepared freshly for each application:

1. Vitamin B_1 (low level), 15 mg. in 500 ml. (10 ml. − 0·3 mg.).
2. Nicotinic acid. Ditto.
3. Vitamin B_1 (high level), 120 mg. in 400 ml. (10 ml. = 3·0 mg.).
4. Nicotinic acid. Ditto.

Applications were made by pipette (10 ml. per pot) and these were watered in after 2–3 hours with ¼ pint water; they were made alternately on the surface and by injection into the centre pot, i.e. one week on the surface, the next below the surface sand.

Seventeen applications in all were made at approximately weekly intervals between April 5 and August 10. Nicotinic acid was obtained from B.D.H., and crystalline vitamin B_1 (aneurin) was purchased from B.D.H. in sealed ampoules at monthly intervals in order to ensure a fresh supply.

It was calculated that each pot at the time of application would contain about 3 litres of solution, and the levels of concentration, therefore, are approximately 0·1 and 1·0 mg. per litre.

Observations on growth.

In all pots the plants germinated on or about March 28 and were at the single-leaf stage 1½ in. high by April 3, two-leaf stage 4 in. high by April 13, and the three-leaf stage 6–7 in. high by April 21. Tillering began on April 27 and was very active early in May. In the early stages up to the middle of April the soil pots were slightly ahead and the plants a better colour, but by the end of April a reverse condition was very definite, the sand pots being much darker in colour and stronger in growth. By May 20 the two had become similar, but after shooting commenced (June 6) the soil pots steadily lost ground and the sand pots became progressively superior from the date of emergence of the panicle June 10–15 towards maturity. During the early stages weather conditions were excellent, but for six weeks in July and early August very dull and wet weather prevailed; in spite of this the plants made slow but steady progress, and a good average crop was obtained. After harvesting on August 16 several pots were washed and their root systems examined. The sand culture root-system was very characteristically different from the soil, being heavier in bulk, much less fibrous in nature, and pale-straw in colour as compared with the dirty greyish-white of the roots in soil. In both series, however, the bulk of the root-system was contained in the top 4 in. of the medium. Under sand culture, the root-system was particularly strong and healthy, and quite free from decay or 'slime'. It may be recorded that the pH of the soil pots drifted only from 7·0 to 7·4 during the season, whereas the sand culture drifted from 6·7 to 9·0; in spite of this, no signs of iron deficiency or chlorosis were ever apparent.

Tillering.

A summary of the tiller count data is shown below:

Average Number of Tillers per Pot of Four Plants. Mean of Six Replicates

Date.	Treatment No.	1	2	3	4	5	6	7	8	9	10	11	12
May 4	. . . .	8	8	8	8	8	8	8	8	8	8	8	8
„ 11	. . . .	12	12	12	13	12	12	12	12	12	12	12	13
„ 25	. . . .	15	14	16	16	16	17	18	16	17	16	17	17
June 15	. . . .	19	19	22	22	20	21	22	21	20	22	23	20
Maturing tillers at harvest	.	16	16	17	16	16	19	17	18	18	18	17	18

During the early stages tillering was equal in all treatments, but later the sand pots tended to be highest. Apart from this, there were no responses to either nicotinic acid or vitamin B$_1$ or the combination of both.

Plant height.

Measurements of plant height (i.e. to the tip of the tallest leaf or top of panicle if showing) were made at intervals, and are summarized in the following table:

Average Height of Plants (cm.). Mean of Twenty-four Individual Tillers per Treatment

Date.	Treatment No.	1	2	3	4	5	6	7	8	9	10	11	12
June 14 .	. . .	54	55	59	64	61	61	64	63	62	64	64	66
,, 22 .	. . .	61	61	71	78	73	72	74	74	73	75	76	77
July 3 .	. . .	79	81	88	93	91	90	90	90	92	93	92	95
Harvest .	. . .	83	82	90	95	92	92	94	92	93	96	93	95

After shooting of the panicle the sand-culture pots were consistently taller than the soil controls, which advantage was maintained up to harvesting, when the sand culture series were on the average about 10 cm. taller; the addition of either organic material separately or together had no effect.

Yield data.

The yield data obtained is summarized below:

Yield Data (gm. per Pot) Mean of Six Replicates

Treatment No.	1	2	3	4	5	6	7	8	9	10	11	12
Panicles . .	15·15	13·12	27·65	26·44	24·84	29·70	27·16	27·35	30·07	27·78	27·23	30·99
Grain . .	10·30	8·86	18·27	17·87	17·36	20·13	18·87	18·65	20·49	18·27	18·60	21·20
Straw . .	61	59	116	99	94	108	91	111	116	110	107	120

Result of Analysis of All Treatments. Mean Grain Yield (gm. per Pot)

Soil controls.	Sand controls.	5	6	7	8	9	10	11	12	General mean.	Standard error.	Significant difference A. B. C.
9·58	18·07	17·36	20·13	18·87	18·65	20·49	18·27	18·60	21·20	17·41	1·397	3·94 3·41 2·79

Result of Analysis of Treatments excluding 1 and 2. Mean Grain Yield (gm. per Pot)

Sand controls.	5	6	7	8	9	10	11	12	General mean.	Standard error.	Significant difference A. B.
18·07	17·36	20·13	18·87	18·87	18·65	20·49	18·60	21·20	18·97	1·456	4·12 3·57

Sig. diff. A is for comparison of any treatments (Nos. 5–12).
,,   ,, B   ,,   ,,   ,, either soil or sand controls *v.* any treated.
,,   ,, C   ,,   ,,   ,, soil controls *v.* sand controls.

EXPERIMENT II: TOMATOES

Experimental.

The experiment, comprising twelve treatments with six-fold replication (72 pots in all), was set up in the greenhouse at Jealott's Hill. In order to conserve space a staging was erected on the centre benches and two pots of each treatment were arranged together, one behind and immediately above the other; these units of two plants were then set out in three random blocks,

there being a top pot and a bottom pot to any particular treatment in each block.[1] The following treatments were included:

1. Soil control.
2. ,,   ,,
3. Sand control.
4. ,,   ,,
5. Sand nutrients + vitamin B_1 0·3 mg./pot weekly.
6. ,,   ,,   + ,,   ,, 3·0   ,,   ,,
7. ,,   ,,   + nicotinic acid 0·3 mg./pot weekly.
8. ,,   ,,   + ,,   ,, 3·0   ,,   ,,
9. ,,   ,,   + vitamin B_1 0·3 mg./pot + nicotinic acid 0·3 mg./pot weekly.
10. ,,   ,,   + ,,   ,, 0·3   ,,   + ,,   ,, 3·0   ,, .   ,,
11. ,,   ,,   + ,,   ,, 3·0   ,,   + ,,   ,, 0·3   ,,   ,,
12. ,,   ,,   + ,,   ,, 3·0   ,,   + ,,   ,, 3·0   ,,   ,,

The soil pots were filled with a compost consisting of 3 parts Berkshire loam, 1 part farmyard manure, 1 part silver sand, 1 part oak leaf mould. Sulphate of potash was added at the rate of 2 lb. per 1½ cwt. of compost. Top-dressing was begun on May 3 (the timed setting of the third trusses) with alternate weekly doses of nitrochalk and sulphate of potash together, and sulphate of ammonia alone, at the rate of ½ oz. of each per pot.

The sand pots (10 in. × 10 in. glazed porcelain) were filled with 28 lb. of Garsides graded 2L silver sand overlying a 2 in. layer of ¼ in. washed pea-gravel.

Planting out was made on March 17 with Stonors M.P. selected plants about 8–9 in. high, which had been raised previously in sterilized soil, and replanted in sand on February 22, after a light washing of the root-system to remove the adhering soil.

Application of nutrients to sand pots.

The following concentrates were prepared:

Concentrate No. 1.		*Concentrate No. 2.*	
$NH_4H_2PO_4$	. 161·8 gm.	$NH_4H_2PO_4$	. 161·8 gm.
KNO_3 . .	. 858·4 ,,	KNO_3 . .	. 469·4 ,,
$(NH_4)_2SO_4$.	. 70·9 ,,	$(NH_4)_2SO_4$.	. 70·9 ,,
NH_4NO_3 .	. 131·4 ,,	KCl . .	. 286·8 ,,
dissolved in 5 galls. water.		dissolved in 5 galls. water.	

(*Minor Elements Concentrate* (gm. in 20 l.)); $LiCl2H_2O$, 0·56; $CuSO_45H_2O$, 1·12; $ZnSO_47H_2O$, 1·12; H_3BO_3, 12·24; $Al_2(SO_4)_3$, 1·12; $SnCl_22H_2O$, 0·56; $MnCl_24H_2O$, 7·84; $NiSO_46H_2O$, 1·12; $Co(NO_3)_26H_2O$, 1·12; TiO_2, 1·12; KI, 0·56; KBr, 0·56; Na_2SiO_3, 8·66; $FeC_6H_5O_73H_2O$, 128·40; $KMnO_4$, 7·48.

The application of nutrients can be divided into two main periods: (*a*) The drip-flow period (March 20–June 30) during which the nutrient solution dripped from a reservoir through a fine jet on to the surface sand of the upper pot, and after flowing through both top and bottom pots was collected in

[1] Each pot was tilted towards the front by inserting a wedge beneath. In the early stages ½ in. fall was allowed, but later in the season this was increased to 1 in., as owing to the position of the outlet tubule the complete drainage of the pot was suspect below the latter figure.

another reservoir on the floor. Once a day the effluent nutrient in the floor-reservoir was transferred back into the top reservoir. This drip-flow system was charged with fresh nutrient at intervals, varying from about a week to ten days in the early stages, down to a daily charge towards the end of the drip-flow period. (*b*) The 'watering-on' period (July 1 to August 28) during which fresh nutrient solution was applied in small doses of 1 pint twice daily by watering-can to the surface sand of each pot, the effluent nutrient, after passing through the pot, being discarded.

(*a*) *The drip-flow period.* (i) March 20–April 24: seven recharges were made (on March 20, 24, April 4, 12, 19, 21, and 24) each consisting of 7 litres[1] of solution having $50N + 50P_2O_5 + 200K_2O$ (mg. per litre), plus a unit amount of minor elements. The solution was obtained thus: 2 pints concentrate No. 2, 100 ml. minor elements concentrate, water to 100 litres adjusted with about 30 ml. of 30 per cent. H_2SO_4 to pH 6. (ii) April 25: one charge of 7 litres of $100N + 50P_2O_5 + 200K_2O$ plus normal minor elements. The solution was as for (i) but concentrate No. 1 was employed. (iii) April 28: one charge of 7 litres of $200N + 100P_2O_5 + 400K_2O$ plus twice the amount of minor elements. The solution was obtained thus: $\frac{1}{2}$ gall. concentrate No. 1, 200 ml. minor elements concentrate, water to 100 litres adjusted to pH 6. (iv) May 3–May 24: 4 charges were made (on May 3, 10, 17, 24), each consisting of 7 litres $300N + 150P_2O_5 + 600K_2O$ plus thrice the amount of minor elements. The solution was obtained thus: 6 pints concentrate No. 1, 300 ml. minor elements concentrate, water to 100 litres adjusted to pH 6. (v) May 30–June 30: daily charge of 7 litres of $100N + 50P_2O_5 + 200K_2O$ plus a normal amount of minor elements. The solution was obtained as for (i) except for the use of concentrate No. 1. During hot weather 1 pint of the effluent nutrient was watered back on to the surface sand with a watering-can during the late afternoon.

(*b*) *The 'watering-on' period.* July 1–August 28. During this period each pot received daily two applications of 1 pint of $100N + 50P_2O_5 + 200K_2O$ plus unit amounts of minor elements. The solution was obtained thus: 2 pints concentrate No. 1, 100 ml. minor elements concentrate, water to 100 litres adjusted to pH 6, with about 30 ml. of 30 per cent. H_2SO_4. The final concentrations of the more important elements in the various nutrient solutions used above are shown in the appendix.

It must be noted that during the drip-flow period, in order to supply sufficient nutrients for growth, either the volume of nutrient or the concentration had to be increased, the unit system having a maximum capacity of only 7 litres for each pair of pots. In the early stages, therefore, increasing the nutrient concentration was tried, since this had an important bearing on the amount of work involved in renewing the solutions (there were 60 sand pots or 30 units to be dealt with), and an increased concentration meant less frequent charging of the solution. Early in May, however, the advent of

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[1] The 7 litres percolated through 2 pots, i.e. one complete unit, for periods (i) to (v).

Blossom-end Rot[1] in the fruit, a purely physiological disorder, was thought to be associated, though not definitely proven, with this increase in concentration of nutrients, and from then onwards, the nutrient concentration was reduced and more frequent charges made. The daily drip-flow system was finally abandoned, after being tried for a month in June, on account of the excessive labour involved in dealing with so many pots. Fortunately the 'watering-on' technique proved both highly satisfactory and particularly easy of manipulation, and there is little doubt that this method could have been used throughout the whole growth period with satisfactory results.

Application of vitamin B_1 and nicotinic acid.

The following concentrates were prepared freshly for each application:

1. Vitamin B_1 (low level), 15 mg. in 500 ml. (10 ml. = 0·3 mg.)
2. Nicotinic acid (low level), 15 mg. in 500 ml. (10 ml. = 0·3 mg.)
3. Vitamin B_1 (high level), 120 mg. in 400 ml. (10 ml. = 3·0 mg.)
4. Nicotinic acid (high level), 120 mg. in 400 ml. (10 ml. = 3·0 mg.)

Applications were made by pipette, 10 ml. per pot, on the surface sand. Eighteen applications in all were made at about weekly intervals between March 27 and August 10. It was assumed that each pot would contain about 3 litres of nutrient solution when applications were made, and the concentration levels, therefore, are approximately in the order of 0·1 mg. and 1·0 mg. Although the treatments were applied to the surface sand in a very small volume of water, the drip-flow and/or watering-on of nutrients would rapidly disperse them through the pot.

Observations on growth.

Growth in sand-culture was very much more rapid than in soil; this effect was first apparent early in April, and was noticeable throughout the whole growing season. During April the sand-culture plants were very heavy in foliage in comparison with the soil ones, and towards the end of April blossom-end rot made its appearance on the sand-culture plants, but disappeared by the middle of June; during the hot weather in May some wilting of the foliage occurred, and as at this period the plants were evaporating 2–3 litres of water a day, a certain amount of surface watering with the effluent nutrient had to be resorted to, and some shading of the house was essential during the hot midday hours. From June onwards, the sand-culture plants made excellent growth and cropped satisfactorily. In spite of a very cool, dull, and wet July, when mildew was troublesome—spraying with Shirlan AG proved to be very effective—the plants still retained a very heavy foliage even at maturity and no sign of wilting or blossom-end rot was again apparent. Unfortunately, the soil plants made very poor growth throughout the whole

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[1] This disorder steadily declined as the season progressed, and by the middle of June had completely disappeared. All treatments (except soil) were affected to the same extent, and in all only about 7 per cent. of the total crop was lost.

season, and cannot be regarded as typical of a normal crop under a high level of manuring. Tomatoes in soil have usually done very well at Jealott's Hill, and the reason for this season's poor results is obscure.

No differences in growth were ever apparent as a result of nicotinic acid and vitamin B_1 applications.

A summary of the truss count data is given below, and illustrates the more rapid growth in sand; treatments, except growth in soil, however, show no effect upon truss development.

Average Number of Trusses in Flower or Fruiting

Date.	Treatment No.	1	2	3	4	5	6	7	8	9	10	11	12
April 12		1·3	1·0	1·7	1·7	1·7	1·7	1·3	1·7	2·0	2·2	1·7	1·7
„ 19		2·2	2·2	2·5	2·7	2·7	2·7	2·3	2·3	2·9	3·0	2·3	2·9
„ 26		2·2	2·0	3·5	3·3	3·5	3·5	3·3	3·2	4·0	3·8	3·5	3·7
May 3		2·7	2·8	4·2	4·2	4·2	3·8	3·8	4·2	4·5	4·0	4·2	4·2
„ 10		3·2	3·0	4·8	4·5	4·7	4·8	4·7	4·8	5·3	5·2	5·0	5·0
„ 17		4·2	3·8	5·7	5·3	5·7	5·5	5·3	5·2	6·0	6·0	5·7	5·8
Finally		9	8	8	9	9	8	8	9	9	9	10	9

After the final cropping in August a few of the plants were washed free from soil or sand and their roots examined; the differences were the same as described for experiment 1.

Yield of fruit.

The yield of fruit from the soil controls was very low, being only 4·3 lb. per plant. Sand culture gave a more satisfactory yield of 6·3 lb. per plant, particularly as only seven trusses could mature satisfactorily. The addition of vitamin B_1 and/or nicotinic acid failed to give any significant increase. All treatments gave very similar production curves for the season, and apart from a tendency to higher yields from the second trusses, fruit production was on the whole comparatively uniform over the first seven trusses. The actual yield data are summarized below:

Total Yield of Ripe Fruit (*lb. per Plant*)

Soil control.	Sand control.	5	6	7	8	9	10	11	12
4·25	6·27	6·07	5·94	6·20	6·72	6·07	6·55	6·90	6·19

Significant differences:

For comparison of any single treatments Nos. 5–12.		= 1·11
„ „ either soil controls or sand controls *v.* any single treatments Nos. 5–12		= 0·96
„ „ soil controls *v.* sand controls		= 0·78

Yield (*gm.*) of Fruit per Truss (*Top Trusses ignored*)

Treatment.	Truss	1	2	3	4	5	6	7
Soil		270	282	281	249	303	272	197
Sand		331	509	388	379	350	382	293
„ +vitamin B_1		313	467	447	309	317	398	338
„ +nicotinic acid		353	495	406	406	410	397	197
„ (vitamin B_1 and nicotinic acid)		351	477	454	391	299	450	323

DISCUSSION

It is evident from the results of these two experiments that tomatoes and spring oats do not respond to applications of vitamin B_1 or nicotinic acid, applied singly at two levels or in all combinations of the two levels. It therefore appears that these two plants are of the type described by Bonner and Greene (1938) as fast-growing, and if these two are at all representative of annual crops (e.g. cereals, root crops, &c.) it seems likely that vitamin B_1 or nicotinic acid either alone or contained in an organic material or manure will be ineffective in increasing the yield of crops of this type. It might be objected that application of the organic materials was only made at intervals and had it been continuous different results might have been obtained. The continuous application was not possible on account of the much larger quantity of vitamin B_1 required and of its high price. It was also felt that it could be reasonably assumed that with an organic-free medium and nutrient solution the technique which was used would have detected any beneficial results. The conclusion must be that if vitamin B_1 and nicotinic acid are necessary for the growth of roots (or other parts of the plant) of spring oats or tomatoes, then these two plants can themselves produce sufficient so to render external applications superfluous.

The results of these experiments give additional evidence that excellent crops can be grown in the complete absence of organic material provided due regard is paid to nutrient and water requirements. The findings of West (1939) are very important and suggest that the growth of slow-growing plants may possibly be accelerated by being grown in association with those of rapid growth.

In a previous publication (Templeman and Watson, 1938) yields of tomatoes of 4·34 lb. per plant when grown in nutrient solution culture were reported. Much better yields have now been obtained and have reached an average of 6·3 lb. per plant[1] in spite of an attack of blossom-end rot; this is of the order of the average grown under commercial soil conditions, although the best growers are reported to raise this average figure up to a maximum of about 10 lb. per plant. The ideal method of application of the nutrient solution to the sand would appear to be the 'continuous drip' one; the simpler technique of watering the nutrient solution daily to the surface of the sand has, however, proved satisfactory.

SUMMARY

1. The application of vitamin B_1 and nicotinic acid each at two concentrations and in the four combinations of these two concentrations has not affected the yield of spring oats or tomatoes when grown in sand and supplied with an entirely inorganic nutrient solution.

[1] Since this paper was sent to press another season's results have become available. In the 1940 experiments where the $100N + 50P_2O_5 + 200K_2O$ mgm./litre nutrient solution was used throughout the full growth period, no blossom-end rot occurred, and an average yield of 11·2 lb. of tomatoes per plant was obtained using this sand-culture technique.

2. The culture of tomatoes in sand with a nutrient solution has given an average yield of 6·3 lb.[1] of fruit per plant.

Thanks are expressed to Dr. S. J. Watson for his continued interest and suggestions in connexion with this work and to Imperial Chemical Industries, Ltd., for permission to publish this paper.

APPENDIX I

Experiment I

Resultant Final Concentration of Elements in Nutrient Solution applied

	No. 1.	No. 2.
Ammonia N	289 mg./l.	174 mg./l.
Nitrate N	711 ,,	326 ,,
Total N	1,000 ,,	500 ,,
P_2O_5	500 ,,	500 ,,
K_2O	2,000 ,,	2,000 ,,
SO_3*	220 ,,	220 ,,
Cl*	— ,,	680 ,,
Total solids (including water-supply)	7·0 gm./l.	5·8 gm./l.

Minor elements (mg./l.): Li, 0·010; Cu, 0·070; Zn, 0·065; B, 0·545; Al, 0·045; Sn, 0·075; Mn, 1·200; Ni, 0·065; Co, 0·055; Ti, 0·170; I, 0·105; Br, 0·095; Si, 0·500; Fe, 6·000; Na_2O, 1·100*.

The pots received water between applications, hence this solution is obviously much stronger than would be advisable under *direct* nutrient culture conditions.

* The water-supply contains in addition, CaO 62, MgO 48, Na_2O 294, SO_3 156, Cl 199, total solids 856 mg. per litre.

APPENDIX II

Experiment I

Composition of Minor Elements Solution (5 × normal)

Weight (gm.) of salts in 20 litres of water: $LiCl2H_2O$, 2·80; $CuSO_45H_2O$, 5·60; $ZnSO_47H_2O$, 5·60; H_3BO_3, 61·20; $Al_2(SO_4)_3$, 5·60; $SnCl_22H_2O$, 2·80; $MnCl_24H_2O$, 39·20; $NiSO_46H_2O$, 5·60; $Co(NO_3)_26H_2O$, 5·60; TiO_2, 5·60; KI, 2·80; KBr, 2·80; Na_2SiO_3, 43·30; $FeC_6H_5O_73H_2O$, 642·00; $KMnO_4$, 37·40.

APPENDIX III

Experiment II

Final Concentrations (mg./litre) of Major and Minor Elements in Nutrient Solutions

Period. N:P:K Ratio	Mar. 20– Apr. 24. 50–50–200	Apr. 25. 100–50–200	Apr. 28. 200–100–400	May 3–24. 300–150–600	May 30– June 30. 100–50–200	July 1– Aug. 28. 100–50–200
Ammonia N	17	29	58	87	29	29
Nitrate N	33	71	142	213	71	71
Total N	50	100	200	300	100	100
P_2O_5	50	50	100	150	50	50
K_2O	200	200	400	600	200	200
SO_3*	22	22	44	66	22	22
Cl*	68	—	—	—	—	—

* The salt content of the water-supply has been given above (Appendix I).

[1] See note on previous page.

APPENDIX III (*continued*)

Minor Elements.

Period. N:P:K Ratio	Mar. 20– Apr. 24. 50–50–200	Apr. 25. 100–50–200	Apr. 28. 200–100–400	May 3–24. 300–150–600	May 30– June 30. 100–50–200	July 1– Aug. 28. 100–50–200
Li	0·002	0·002	0·004	0·006	0·002	0·002
Cu	0·014	0·014	0·028	0·042	0·014	0·014
Zn	0·013	0·013	0·026	0·039	0·013	0·013
B	0·109	0·109	0·218	0·327	0·109	0·109
Al	0·009	0·009	0·018	0·027	0·009	0·009
Sn	0·015	0·015	0·030	0·045	0·015	0·015
Mn	0·240	0·240	0·480	0·720	0·240	0·240
Ni	0·013	0·013	0·026	0·039	0·013	0·013
Co	0·011	0·011	0·022	0·033	0·011	0·011
Ti	0·034	0·034	0·068	0·102	0·034	0·034
I	0·021	0·021	0·042	0·063	0·021	0·021
Br	0·019	0·019	0·038	0·057	0·019	0·019
Si	0·100	0·100	0·200	0·300	0·100	0·100
Fe	1·200	1·200	2·400	3·600	1·200	1·200
Na₂O*	0·220	0·220	0·440	0·660	0·220	0·220
Weight of salts in gm./litre* including minor elements and water supply	1·36	1·48	2·09	2·71	1·48	1·48

* The salt content of the water-supply has been given on p. 145.

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The Cytology of the Cricket Bat Willow
(Salix alba var. caerulea)

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With twenty Figures in the Text

INTRODUCTION

THE difficulties which confront the grower of timber suitable for the manufacture of cricket bats have been clearly set forth by Burtt Davy (1932). It appears to be generally agreed that *Salix alba* var. *caerulea* Sm. (the close-bark or blue willow) furnishes the best quality of bat timber, though usable clefts can be obtained from the wood of *S. alba* L., *S. fragilis* L., and from various hybrids between them. *S. fragilis* yields timber of inferior quality except perhaps from the basal region of the trunk. Whilst *S. alba* and some of the *alba-fragilis* (*viridis* Fries.) hybrids are better in this respect, *S. alba* var. *caerulea* is far superior to any.

Burtt Davy in a critical systematic study of *S. alba* var. *caerulea* has found that the tree displays a pyramidal habit consequent upon rapid and vigorous growth. The individuals recognized with certainty as being var. *caerulea* are all female, though Burtt Davy claims to have found males. In the female form the stems are straight, the branching is developed in an upright manner, and the bark is close. The peduncles and catkins are short, the bracteoles are also short and rounded, and the carpels possess no definite style.

The question whether these features may be regarded as characters with a varietal value or merely as expressions of physiological differences is not beyond dispute, though the evidence supports the former view. That the development of an upright form is not due to overcrowding is abundantly demonstrated, as Burtt Davy points out, by an examination of typical specimens growing in open hedgerows in Essex and East Anglia, where such conditions prevail as would not discourage the tree from taking on a lax and spreading habit. Shoots from a specimen of *S. caerulea* planted in widely separate localities and on different types of soil also produce trees which remain pyramidal in habit at least up to the age of fifteen years. *S. alba* var. *caerulea* appears to differ from *S. alba* principally in the upright habit and rapidity of growth, though some maintain that glabrescence of the under surface of the *caerulea* leaf is also typical.

Salix fragilis L. is sufficiently distinct from *S. alba* to warrant its assignment to a different sub-group (Linton, 1913). It is lax and spreading in habit, and

the stems have rough bark, the twigs readily breaking off at the base. The leaf-blades are glabrescent (except when very young) and coarsely serrated; the ovaries are short-pedicelled and attenuate into a long style.

The *Salix viridis* group of hybrids exhibits a wide range of characters, approaching to a greater or lesser degree one or other of the parents, *S. alba* and *S. fragilis*. Some of the *alba-fragilis* segregates are of some value to the growers of cricket bat timber; Burtt Davy, however, has stressed the fact that groups of characters do not necessarily remain associated, since certain individual characters often interchange to form new combinations. The determining factors for wood characters evidently undergo genetic crossing-over. Thus matroclinous *S. viridis* segregates from an *S. alba* var. *caerulea* × *S. fragilis* cross are not necessarily good from the point of view of timber quality, and forms leading towards the *S. fragilis* parent indeed may often be better in this respect.

It is very unfortunate from the point of view of the growers of bat timber that the above distinctions associated with mature *alba*, *fragilis*, and *viridis* types are not manifest when dealing with young trees propagated from sets. For this reason a large proportion of the sets planted fifteen to twenty years ago have been found either to be worthless or of such poor quality as to impose severe financial loss upon the planters. Nor do other characters help much. For instance Bancroft (1934) after studying the stomatal characters has laid emphasis on the fact that 'external leaf characters do not provide consistent and reliable diagnostic data unless used with extreme discretion'. Stomatal counts are probably of value only in the case of mature individuals of different species and varieties grown under identically similar conditions. Thus *S. alba* and its variety *caerulea* have, on the average, respectively 114 and 113 stomata per square millimetre, and *S. fragilis* about half this number. *S. viridis* var. *eleyensis* (Burtt Davy) apparently does not differ materially in this respect from *S. alba*. Nor is the glabrescence of the under side of the leaf of the cricket bat willow a reliable distinguishing character, since old leaves may display varying degrees of pubescence.

Addressing the Forestry Sub-section of the British Association in 1933, Pratt (1934) remarked: 'In these days of over-production, we find in the case of many articles that, while the best quality still fetches a fair price, second quality material is practically unsaleable . . . this is the position with the cricket bat willow.' There is obviously a need for some critical method of distinguishing *S. alba* var. *caerulea* from unsuitable though nearly related forms, and this has prompted the present cytological line of approach.

A preliminary report by the present writer upon the cytology of the cricket bat willow group appeared some time ago (Wilkinson, 1934). In this it is indicated that the somatic complements of *S. alba* and *S. alba* var. *caerulea* differ from one another in the number of chromosome satellites, and from *S. fragilis* and the *viridis* hybrids in chromosome morphology. The investigation has since been considerably extended and chromosome measurements have been carried out. The value of the results obtained is discussed later.

TECHNIQUE

Most of the known types and material of unknown ancestry (later referred to as 'unknown') for this investigation were obtained from Oxford and from the estates of Mr. Amos, Wye, Kent, and of Lord Selborne, Liss, Hants, through the kind collaboration of Dr. Burtt Davy. Mr. Hutchinson has kindly supplied cuttings from Long Ashton, Bristol. In addition to this a fair amount of material collected in Durham and Northumberland has been examined, and one specimen from Kew has been investigated.

Only the somatic material has yielded results likely to be of any practical use. Although pollen mother cells have been examined in the case of some of the hybrids, and meiotic stages have been seen in the ovule, the findings are largely of theoretical interest, and are reported here only briefly.

Bat willow sets and cuttings from the other relevant types belonging to the *S. alba* and *S. fragilis* groups will root very readily in water. All cuttings dealt with were rooted in the cool section of the greenhouse. The tips were fixed after having emerged about one inch from the root-papillae. As mentioned in the preliminary paper (Wilkinson, 1934) a considerable range of fixatives was tried, but none proved so uniformly satisfactory as Langlet's modification of Navashin's Fluid, the use of which consequently has been retained throughout. The catkins of these species, unlike those of the Capreae and most other Salix groups, are sufficiently free from siliceous hairs to allow of treatment upon the microtome without dissecting out the anthers and ovules. Carnoy-Langlet fixation was employed for the meiotic material, followed by the routine paraffin technique. The aceto-carmine method of making temporary smears met with little success, as the ever-troublesome cell background became too intense very shortly after the slides were prepared.

Substances of the tannin class present in the cytoplasm have caused a great deal of trouble in connexion with the staining of the preparations. A clear cytoplasmic background is pre-eminently required for the study of large numbers of small chromosomes, and of the extremely minute satellites encountered in the Salices. This necessitates light staining. On the other hand, the satellites themselves require as intense and opaque a stain as possible, and from this standpoint Heidenhain's iron-alum-haematoxylin is unsurpassed. This stain as ordinarily employed greatly emphasizes the willow cell background, and it was soon realized that some departure from standard practice would be necessary to fulfil the first requirements. The modifications employed in all the somatic preparations for this investigation were simple, consisting in the main of drastic variation of the mordanting and staining periods. Mordanting in 2 per cent. iron alum was short (1 hour), and staining in haematoxylin occupied not less than three nor more than five days. With many tips the latter period, determined by trial and error, was most suitable. Thus, though the chromatic material, in comparison with other cytoplasmic inclusions of Salix species, exhibits marked preferential absorption of haematoxylin, the rate of absorption is slow. In order to retain the low level of background attained by

this means, the counterstain customarily employed (viz. Orange G in the clove oil used as clearing agent) was omitted from the technique.

The best preparations for observing the association between the pachytene threads and the nucleolus were obtained from iron aceto-carmine smears. Unfortunately the smears deteriorate very quickly, and the drawings for Figs. 15 and 16 had to be made after the slides had been not more than a day or two in the refrigerator. Concentrated iron aceto-carmine was used on both pollen and embryo-sac mother cells, and the gently heated preparations were pressed out under the cover-glass. When ovules were being treated, this procedure had to be repeated two or three times. For a short time the prophase figures obtained by this method remained exceptionally clear, and superior to those obtained by the use of Carnoy-Langlet fixative, which tended to cause collapse in the region of the nucleolar membrane.

The somatic metaphase chromosomes of these Salix species under discussion are short, none exceeding 1.75μ, and by examining sufficient material cut at 6μ it was found possible to obtain cells with perfectly flat metaphase plates. It was therefore decided to take measurements from camera lucida drawings made at a projected magnification of 4,500, avoiding the use of Kagawa's projection method, which in any case would be difficult to apply in the case of chromosomes so short as this. It is generally recognized (see, for example, Navashin, 1934) that an accuracy greater than 0.1μ cannot be attained in chromosome measurement, and this would constitute a high percentage of tolerance in the case of even the longest chromosome of the complement; on this account it was felt that a better comparative idea of size relationships would be obtained by assigning the chromosome groups into length classes differing by 0.25μ. No attempt was therefore made to measure individual chromosomes with great accuracy. Chromosome size also varies somewhat (*a*) from tissue to tissue, (*b*) with the fixative employed, and (*c*) according to whether the roots are taken from embryonic or adult material (Navashin, 1934). In this investigation (*b*) and (*c*) always remained constant, since the same method of fixation was always employed on roots from cuttings, and all that remained was to consider chromosome complements of cells from the peripheral layers of the root cortex.

All except one of the drawings shown were made at a magnification of 4,500, using a Leitz 1/12 oil-immersion objective and 20 × periplan ocular, and a Bausch and Lomb camera lucida. The somatic complements have been carefully checked for chromosome morphology with the same objective and a binocular attachment carrying Leitz 15 × eyepieces; by this means satellites can be recognized after practice with reasonable certainty.

THE SOMATIC METAPHASE CHROMOSOMES

1. *The* S. alba *complement.*

Remarkable uniformity has generally been found, amongst the somatic complements examined of *S. alba* and its varieties, in material originating

from widely different localities. The somatic chromosome number is 76, and the chromosome lengths vary from slightly less than 0·5 to approximately 1·75 μ. A certain amount of 'somatic pairing' (2–8 pairs) appears on every plate.

The great majority of the chromosomes show obvious constrictions. On every *S. alba* plate the two longest chromosomes have secondary constrictions. Except in the case of one *S. alba* var. *caerulea* examined, there are eight short chromosomes without evident constrictions, and in them the spindle fibre insertion is presumably terminal; no sign of a 'sub-terminal knob' has been seen. The number of satellites appears to be a character of some importance, for, although it is true that in some otherwise satisfactory plates the satellites have been indeterminate, the great majority of *S. alba* plates other than *S. caerulea* have without doubt shown at least four satellited chromosomes, two with constrictions (apart from those separating the trabants, if these are true constrictions), the others without median constrictions (e.g. Fig. 1). On the other hand, in the bat willow plates only two of the chromosomes have yet been observed to have satellites, and these chromosomes show primary constrictions. These satellites are extremely minute, and their observation requires practice coupled with very careful technique.

One somatic complement from Long Ashton material (*S. caerulea* E, Fig. 3) proved exceptional in having four long chromosomes with constrictions in addition to those associated with the fibre insertion region. This material also showed the six unconstricted chromosomes already mentioned. In addition to the longest pair of chromosomes, two chromosomes in the penultimate length class (viz. 1·25–1·5 μ) can invariably be readily observed in the *S. alba* complement, and occasionally four of these are present. On the whole there is no significant difference between the numbers of chromosomes found in the various length classes for any *S. alba* complement.

2. *The* S. fragilis *karyotype.*

The complements of *S. fragilis* differ only in detail from those of *S. alba.* The distribution of chromosomes in the various length classes is substantially the same, except that the two longest chromosomes with secondary constrictions of *S. alba* are missing from the *S. fragilis* complement. Eight short unconstricted chromosomes are present, as in the *S. alba* complement, two of which are satellited. Two additional satellited chromosomes with constrictions can also be observed.

Exceptional features have been presented by *S. fragilis* var. *Basfordiana* from Kew and var. *Basfordiensis* (henceforth referred to here as *Basfordiana*) received from Long Ashton (e.g. Fig. 5). In complements from each of these long, secondarily constricted chromosomes in the highest length class of the *S. alba* karyotype appear. Thus, excluding the bat willow, there is nothing on grounds of somatic chromosome morphology to distinguish these varieties of *S. fragilis* from *S. alba.*
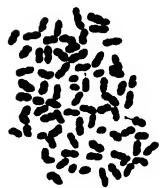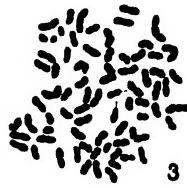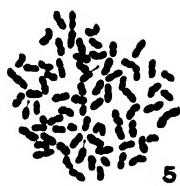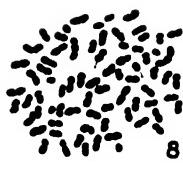
3. *Selected hybrids.*

Somatic complements from *Salix viridis* var. *roystonensis* from Wye (Fig. 6), *S. viridis* var. *eleyensis* from Liss (Fig. 7), and of matroclinous and patroclinous *caerulea* × *Basfordiana* crosses from Long Ashton (e.g. Fig. 8) are shown.

The *roystonensis* and *eleyensis* crosses immediately reveal their hybrid nature by the presence of a single long chromosome with both primary and secondary constrictions. There are only two satellited chromosomes to be seen, and these are unlike, one being constricted and of medium length, the other relatively short and unconstricted. The assortment in size classes is observable in good plates without resort to chromosome measurement. The eight unconstricted chromosomes, for example, are seen to be much shorter in the hybrid than in either of the pure parental species.

It should be mentioned that amongst the *S. roystonensis* material, one type was found to have a *caerulea* complement. The presence of some bat willow material amongst the batch of *roystonensis* cuttings had already been suspected.

The *caerulea-Basfordiana* hybrids show somatic complements of considerable interest. Two long and secondarily constricted chromosomes are present, which is what one would expect, paying due regard to the deviation of *S. Basfordiana* in the direction of the *S. alba* type complement. Again, however, only two satellited chromosomes are present; a careful analysis of exceptionally good plates shows these to be unequal, one bearing a visible constriction, and the other without this feature.

In all these hybrid complements the distribution of chromosomes in size classes is substantially the same and different from those of the typical *S. alba* and *S. fragilis* karyotype. There appears to be an overflow of chromosomes into the length classes at the extremes of the scale; thus the number of chromosomes less than $0.5\ \mu$ long increases from about four to approximately twelve, and there is an unmistakable tendency towards increase in number in the two size classes above $1.25\ \mu$. On the other hand, the class in the uncontaminated species with the highest number, namely $0.75-1\ \mu$, suffers reduction to the extent of about ten chromosomes. In all features except the inequality of the satellited individuals and the distribution of chromosomes for length, the somatic complements of the *caerulea* × *Basfordiana* hybrids, whether matroclinous or patroclinous, appear to be indistinguishable from the typical bat willow complement.

4. *Selected 'Unknowns'*

(i) *S. 'alba' (Stamfordham, Northumberland*; Fig. 9). The cuttings examined were not quite typical for *S. alba*, the leaves tending to be narrowly

FIGS. 1–14. Somatic metaphase chromosomes. Fig. 1. *Salix alba*, 777. Fig. 2. *S. caerulea*, 26262. Fig. 3. *S. caerulea* E (from Long Ashton, Bristol). Fig. 4. *S. fragilis* (Jesmond Dene, Newcastle-on-Tyne). Fig. 5. *S. Basfordiana* (Kew). Fig. 6. *S. viridis* var. *roystonensis*. Fig. 7. *S. viridis* var. *eleyensis*. Fig. 8. *S. caerulea* var. *Basfordiana* (matroclinous). Fig. 9 *S. 'alba'* (Stamfordham, Northumberland). Fig. 10. 27342 (Oxford). Fig. 11. 24686 (Oxford). Fig. 12. 27315 (Oxford). Fig. 13. 'Blown Up' (Oxford). Fig. 14. 'Big Tore' (Oxford).

lanceolate rather than obovate-lanceolate, and the silky pubescence appearing to be somewhat less than it should have been (these characters, it must be admitted, vary according to the time when the cuttings are taken). An examination of the somatic metaphase would suggest that the specimen is of hybrid nature. Only one secondarily constricted long chromosome is present, there are only two satellited chromosomes, which are unequal, and there is a preponderance of small chromosomes. The last feature is obvious at a glance, and it seems probable that the tree is a hybrid between *S. alba* and *S. fragilis.*

(ii) *Unknown No. 27342, from Oxford* (Fig. 10). A consideration of the distribution of the chromosomes, together with the fact that two secondarily constricted chromosomes are present, establish this as probably an *alba*, with *Basfordiana* as a remote possibility. Only two satellited chromosomes are present, however, and since these are constricted and equal in size, the plant may be *Salix caerulea.*

(iii) *Unknown No. 24686, from Oxford* (Fig. 11). The best plate displayed a single long chromosome with a secondary constriction, and two satellited chromosomes, one medianly constricted and the other apparently not constricted; measurements showed that more small chromosomes were present than in a pure species. A hybrid between *S. alba* and *S. fragilis* is therefore indicated.

(iv) *Unknown No. 27315 (Oxford,* Fig. 12). The measurements of the somatic chromosomes showed a class distribution similar to that of a typical *S. alba* complement. Two satellited small pairs were found, one pair constricted and the other not; the plant is therefore probably *S. alba.*

(v) *Unknowns described as 'Blown up' (Oxford,* Fig. 13) *and 'Big Tore'* (*Oxford,* Fig. 14). By the relative abundance of chromosomes in the lower length class, both these trees exhibit hybridity. There is no increase in the numbers of chromosomes at the upper end of the scale. It may be that this material represents an F_2 generation, in which case no exact analysis can be made at present.

(vii) *Unknown No. 11, 'with Armillaria mellea' (Oxford,* Fig. 14). From a consideration of the numbers of chromosomes in the size classes, and the fact that the requisite two satellited pairs and the secondarily constricted pair are present on the complement, the material is probably *S. alba.* It was difficult to decide whether or not there was an additional pair with secondary constrictions in the penultimate length class. If this were the case, a further *alba* variant of the same order as *S. caerulea* E (Bristol) would be established.

The Meiotic Material

Only the Long Ashton cuttings have given meiotic stages worthy of record. The phases described here were observed in two plants of *S. caerulea,* in *S. fragilis* var. *Basfordiana,* and in two individuals from a *caerulea-Basfordiana* cross.

A great many catkins of different sizes both before and after emergence from the winter bud have been cut, and it has been found that the size of a catkin before maturity affords little indication of the meiotic stages likely to be encountered. Indeed, it often happens that a given catkin will show nearly all the anthers to contain pollen mother cells at prophase, the rest having nothing but pollen tetrads, with no intermediate gradation of stages. It appears that in the willows of these groups the prophase of meiosis is initiated during the winter, while the very young catkin is still in the winter bud, and is suspended until early spring, when the succeeding reduction divisions proceed very rapidly. Hence, especially in the female, success in obtaining meiotic stages is largely a matter of chance.

'Smearing' methods using iron aceto-carmine gave satisfactory prophase preparations. There were too many chromosomes present for the recognition of individuals to be possible, but the association of the satellited chromosomes with the nucleolus at pachytene was fairly well seen. In the two *S. caerulea* and in the *fragilis-caerulea* hybrids, the two satellited chromosomes were attached, (e.g. Fig. 15), and in *S. Basfordiana* the attachment of two pairs was observed (Fig. 16). A twin embryo sac in prophase was seen in *S. caerulea* A (Fig. 15).

Diakinesis was observed in all types and exhibited the same features throughout. The great majority of bivalents showed a cross-shaped configuration, suggesting the formation of random interstitial chiasmata. In a few of the pairs terminalization was apparent, but at least two of the longest bivalents exhibited a pair each of interstitial chiasmata, with the ends of the chromosomes free (see Fig. 17).

In *S. Basfordiana* (male) and the males of the two *caerulea-Basfordiana* hybrids, at least 40 per cent. of the pollen appeared to be degenerate, microcytes often being encountered. At metaphase I in all of these, two, three, or four univalents were often seen on the spindle or in the cytoplasm. Sometimes, however, the symmetrical arrangement of some of these eliminated univalents at early anaphase suggested precocious anaphasic separation (Fig. 18, one pair). From three to five quadrivalents were regularly seen at first metaphase.

The only example of an anaphase met with in *S. caerulea* suggested that lagging occurred on the spindle of the separating bivalents. The same thing was observed with certainty in many of the first division anaphase figures of *S. Basfordiana* and the hybrids. It is probably related to the formation of more than one interstitial chiasma followed by terminalization between some of the longer bivalents, the great majority of the separating chromosomes not being delayed by the terminalization process. There was a suggestion of drawn-out connexions between some of the separating chromosomes (Fig. 19).

The second division in the pollen mother cells appeared on the whole to be fairly regular. Again, univalents were occasionally excluded, remaining in the cytoplasm or on the spindle towards the poles (Fig. 20).

DISCUSSION

All the species under consideration are tetraploids on the 19-base series for Salix. No deviation from the number 76 for the somatic chromosome
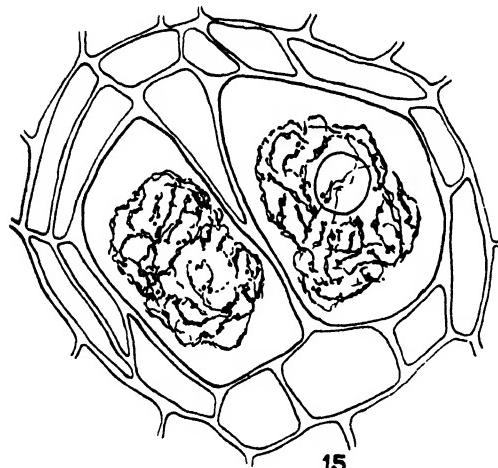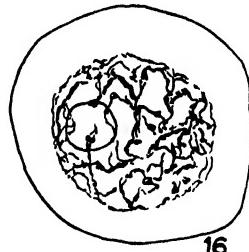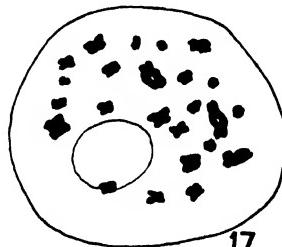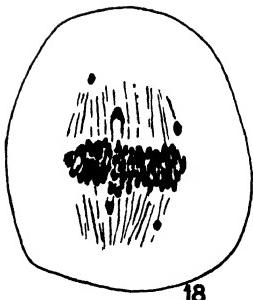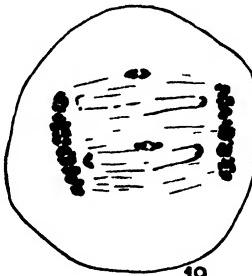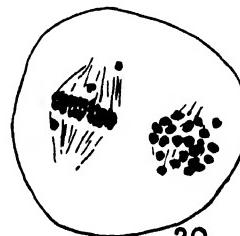
FIGS. 15–20. Stages in meiosis. Fig. 15. *S. caerulea* A (Long Ashton). Twin embryo sac showing association between the nucleolus and one pair of satellited chromosomes. Fig. 16. *S. Basfordiana* (Long Ashton). Pollen mother cell showing attachment of two satellited pairs to nucleolar membrane. Fig. 17. *S. caerulea* × *Basfordiana*. Diakinesis. Fig. 18. *S. Basfordiana*. Pollen mother cell at early anaphase, showing elimination of univalents. Fig. 19. *S. Basfordiana*. Anaphasic lagging of longer homologues. Fig. 20. *S. Basfordiana*. Irregularities of the second meiotic division in typical pollen mother cell.

complement has been found in any root-tissue examined. This is rather remarkable in view of the fact that the incidence of root fasciation is somewhat high, a type of abnormality well known to be conducive to the occasional doubling of somatic complements.

The fact that the somatic chromosomes of several members of the Sali-

caceae show a range of size was noted in 1924 by Blackburn and Harrison, and in 1936 by Müntzing. A formal record of the length of each chromosome is likely to prove of little use in the idiograms here considered, for the smallness of the chromosomes renders the error too great. At a projected magnification of 4,500 diameters, inaccuracy by so much as the thickness of an ordinary pencil line represents an error of about 0·1 μ, and for the average chromosome of the complement this amounts to more than 10 per cent. The matter becomes very important in relation to the changes in length, induced by hybridity, of the chromosomes of each parental complement. Such changes have been found by Navashin to occur in Crepis hybrids, to the extent of 16 and 14 per cent. for the respective parental complements in a typical case, namely that of *C. capillaris* × *C. neglecta* (Navashin, 1934).

When a method of assigning chromosomes to size classes is adopted, the limits of each class can be so chosen as to coincide with the length of only a small number of chromosomes, which is achieved in practice for the material under consideration by taking classes which ascend in steps of 0·25 μ. That this is so is seen by the close agreement of the numbers in each class for the twelve best metaphase plates of each type studied. The numerical data offered (see Table) are only of qualitative value, but it is believed that they are sufficient to demonstrate, for instance, the alteration in length in the members of two haploid sets in a hybrid complement. For a well established species, possessing nuclear symmetry, one would anticipate the number of chromosomes in each size class to be even, but in an F_1 hybrid the numbers may be odd or even, according to whether the difference in size between corresponding chromosomes in each complement is sufficient to relegate one of the chromosomes into another class.

The results of this Salix study, as with Pierce's (1936) work on a sterile Viola species-hybrid, can be interpreted on the basis of Navashin's views on 'regular amphiplasty'. Working with twenty-one hybrids between various Crepis species, M. Navashin has established that in thirteen cases the satellites of one of the parental complements disappear. Thus, to take one of the clearest instances, when *C. capillaris* and *C. tectorum* hybridize, the satellite of *tectorum* disappears and that of *capillaris* remains in the heterozygotic complement. The missing satellite presumably fuses with the end of its associated chromosome. Thus either the whole, or possibly some part, of the *capillaris* haploid complement present appears to induce alteration merely of the satellited member of the other complement, and the phenomenon is consequently spoken of as 'differential amphiplasty'. Another type of controlling influence operates when all the chromosomes of each parental complement undergo modification in length, as in the *capillaris* × *neglecta* hybrids already cited. This type of change is called by Navashin 'neutral amphiplasty'.

An analysis of the *S. viridis* complement, both of var. *roystonensis* and *eleyensis*, would support the view that each of these types of regular

Salix Species, Varieties and Crosses
Typical Assortment of Chromosome Numbers into Length Classes

Plant.	0–0.25	0.25–0.5	0.5–0.75	0.75–1.0	1.0–1.25	1.25–1.50	1.50–1.75
Alba-type:							
Salix alba 26261 (Oxford)	—	4	22	36	10	2	2
„ *alba* 777 (Oxford)	—	4	24	36	8	2	2
„ *alba* F (Bristol)	—	2	24	36	10·	2	2
S. alba var. *caerulea* 26262 (Oxford)	—	2	22	34	12	4	2
„ „ *caerulea* E (Bristol)	—	2	22	32	12	4	4
„ „ *caerulea* A (Bristol)	—	4	22	34	10	4	2
„ „ *caerulea* 711 (Oxford)	—	2	26	34	10	2	2
„ „ *caerulea* 26264 (Oxford)	—	4	26	32	10	2	2
Fragilis-type:							
Salix fragilis (Urpeth, Co. Durham)	—	2	26	34	10	4	—
„ *fragilis* 26254 (Oxford)	—	4	28	30	10	4	—
„ *fragilis* (Ox Close, Co. Durham)	—	2	26	32	10	6	—
„ *fragilis* (Jesmond Dene N/C)	—	2	22	36	12	4	—
„ *fragilis* var. *Basfordiana* (Kew)	—	2	24	34	10	4	2
„ „ (Bristol)	—	2	24	32	12	4	2
Known hybrids:							
Caerulea × *Basfordiana* C (Bristol)	4	4	24	24	12	4	4
„ „ D (Bristol)	4	4	22	26	12	4	4
Viridis var. *roystonensis* 100/33 No. 5	4	6	22	22	12	6	4
„ „ 100/33 No. 7 (Oxford)	4	4	20	24	14	6	4
Viridis var. *eleyensis* 104/33 No. 4 (Liss)	6	6	24	20	12	5	3
„ „ 104/33 No. 8	4	4	22	24	12	6	4
„ „ 104/33 No. 10	6	4	22	24	12	6	2
„ „ 104/33 No. 17	4	4	24	24	12	4	4
Unknown for test:							
'alba' (Stamfordham)	4	4	26	24	11	4	3
27342 (Oxford)	—	2	24	32	12	4	2
24686 (Oxford)	2	6	24	24	12	6	2
27315 (Oxford)	—	4	22	36	10	2	2
'Blown Up' (Oxford)	4	4	26	28	10	2	2
'Big Tore' (Oxford)	6	6	24	28	8	2	2
No. 5 (Oxford)	—	4	30	28	10	4	—
No. 11 'with *Armillaria mellea*' (Oxford)	—	4	24	32	10	4	2

amphiplasty plays its part: there is an apparent suppression of two of the four satellites which one would otherwise expect to see, having in mind the presence of two unequal pairs of satellited chromosomes in the complements of *S. alba* and *S. fragilis*. From a consideration of this cross alone, however, it is impossible to decide whether the haploid complement of *alba* (possessing one unlike satellited pair) is associated with the suppression of the satellites of the morphologically identical satellited pair of the haploid complement of *fragilis*, or vice versa. The *caerulea* × *Basfordiana* crosses (from Bristol),

however, are more decisive. The *S. caerulea* egg-cell has a single satellited chromosome with a median constriction, and the *S. Basfordiana* gamete an apparently identical one, together with one smaller unconstricted and satellited chromosome. If the former 'suppresses' the satellites of the latter, then only one satellited chromosome, and that bearing a median constriction, will appear in the entire hybrid complement. On the other hand, the 'suppression' of the *S. caerulea* satellite by the *S. Basfordiana* complement will result in an unequal satellited pair in the zygote. The latter appears to be the case according to the observations carried out up to the present.

Still more striking is the tendency towards increase in the numbers of chromosomes in the size classes at the upper and lower ends of the scale. The extent of the increase is of the same order throughout the range of known F_1 hybrids examined. Without doubt, the marked increase in numbers of chromosomes in the lowest class for length can be correlated with neutral amphiplasty, and this is also probably true for the highest and penultimate classes. There is no certain evidence to indicate which of the parental complements undergoes shortening of all its members and which exhibits increase in length; the only members of the idiogram which would afford such evidence are the secondarily constricted chromosomes of the longest class. In the case of the *caerulea × Basfordiana* crosses, one of these would be received from each parent, and even if one of the pair in the hybrid were longer than the other, it would be impossible to name the parent from which it came. Nor is change in length of the satellited pair (the *S. Basfordiana* origin of these having already been established) sufficiently conclusive. Measurement would suggest that these two chromosomes increase in length to the extent of about $0 \cdot 1 \, \mu$; but since length determinations on such small chromosomes cannot be guaranteed as closely as this, the result must be discarded. Again, in one of the *viridis* hybrids (104/33, No. 4; see Table) two of the longest chromosomes with median constrictions increase sufficiently to justify inclusion in the highest length class, while there is suspicion of shortening of the secondarily constricted one. On the other hand, another hybrid (104/33, No. 8) shows a similar increase in some of the long singly constricted chromosomes, together with *lengthening* of the singly constricted individual. In view of this, no definite conclusion can be drawn.

In the nomenclature here adopted the very long thread which separates the minute satellite from its chromosome is, for the sake of convenience, not regarded as a true constriction. 'Secondary' constrictions are those occurring along the main chromosome body additional to the spindle-fibre constriction. The existence of primary and secondary constrictions has been demonstrated in an extremely wide range of species, notably by S. Navashin, Delaunay, Taylor, Newton, Sharp, and many others. The median constrictions in the majority of the chromosomes of the somatic complement appear to be related to the number of interstitial chiasmata formed, judging by the large number of cross-shaped bivalents at diakinesis; and further correlation is apparent in

the occurrence of two interstitial chiasmata in the longer pairs, presumably the chromosomes with secondary constrictions. In view of the fact that several of the shortest chromosomes are apparently entirely unconstricted, however, it is difficult to agree with Taylor's (1926) generalization that 'there is always at least a sub-spherical knob about the basis of which attachment seems to be effected'.

It has been pointed out by Lawrence (1931) that certain genera such as Salix, Vitis, Gossypium, &c., with high basic numbers are secondary polyploids. If secondary association is accepted as a criterion of homology, this is supported for the Albae and Fragiles groups of Salix. At the first metaphase of meiosis, secondary association of the bivalents was pronounced in *S. Basfordiana* and the male progeny of the two *caerulea* × *Basfordiana* crosses studied. An examination of the somatic metaphase plates shows in the case of all material examined, both 'uncontaminated' and hybrid, the presence of from two to six pairs of identical chromosomes lying side by side along their entire length. The chromosomes displaying this somatic pairing usually belong to the small or medium length classes, and, as has already been reported by the writer (1934), the smallest pair of chromosomes of the *S. alba* complement frequently appear in secondary association. As the chromosomes of Salix are much smaller than the average, even for the majority of dicotyledonous plants, additional support in a negative sense is lent to the suggestion of Lawrence that long chromosomes do not show secondary association because (*a*) relatively large masses of non-homologous chromatin would then be in close proximity, and (*b*) there would be less space for free movement. It is difficult, however, to see why homologous chromosomes, repelling one another at meiosis and only being held at first metaphase by the chiasmata, should yet remain in close association on the somatic metaphase plate; and, for that matter, there is evidence of the non-homologous association of the ends of chromosome on the plate which shows somatic pairing. In addition, quadrivalents found at the first metaphase in pollen mother cells of all male plants examined afford evidence in favour of the evolution of Salix by so-called secondary polyploidy. It is also significant that Håkansson (1933) has observed polyvalent associations in *S. phylicifolia* ($n = 57$), and in *nigricans* × *phylicifolia* (both 57-paired species) and other hybrids.

All the somatic plates show that there is a tendency for the ends of the chromosomes to come together. This has also been observed amongst the Salicaceae in *Populus tremula* by Müntzing (1936). There is often a very strong suggestion that there are connexions between certain chromosomes. Collapse due to bad fixation has often been suggested as the cause of this. However, in view of the necessity for careful fixation and the wide range of fixatives tested in the present investigation, the effect is not likely to be due to an artefact. Probably it is the result of association between groups of non-homologous chromosomes on the somatic plate. The coming together of the chromosome ends would take place with relative ease in the case of the very

small Salix chromosomes, as there is undoubtedly sufficient space on the plate for their unhindered approach. It is hoped subsequently to deal in greater detail with the question of secondary pairing for a range of Salix species and hybrids.

The work of Blackburn and Harrison (1924) on *S. Basfordiana* and of Burtt Davy (1932) on *S. caerulea* has raised the question whether these are truly varietal forms of *S. fragilis* and *S. alba* respectively, or whether they are really of hybrid status. There can be little doubt that they have evolved by hybridity, exhibiting, in common with the other members of the Albae and Fragiles, non-conjunction of univalents at first meiotic metaphase and lagging at anaphase. Some of the apparent untidiness of the Division I anaphase is, however, probably due to the later separation of the long chromosomes with secondary constrictions, on account of the formation of more than one interstitial chiasma.

The satellite number would, at first glance, suggest that the cricket bat willow is a hybrid. The fact that only one chromosome pair is associated with the nucleolus, as in *Zea mays* (McClintock, 1929), supports the view that there are but two satellites present in the complement. Similarly, there are four satellites in the somatic plates of *S. Basfordiana*. The condition of *S. caerulea* regarding satellites would appear to be similar to that of the *alba-fragilis* crosses, which also have two satellites.

However, *S. caerulea* is probably correctly regarded as a variety of *S. alba*, for the nucleus is symmetrical. The satellited chromosomes are both constricted and apparently equal in length. On the other hand, in the hybrid complements just mentioned, one of the satellited chromosomes is much smaller than the other and has no median constriction. Further, the assignment of chromosomes to length classes for *S. caerulea* is not significantly different from that of the typical *S. alba* complement. Similar arguments apply in the case of *S. fragilis* var. *Basfordiana*. It thus seems fairly safe to assert at least that these have not arisen recently as hybrids.

Both the *alba* and *fragilis* species and varieties are unquestionably amphidiploid, i.e. have originated by the crossing of two simpler species followed by doubling of the chromosome complement. Probably karyophylesis has progressed as follows: starting with two hypothetical $2n$ species, each with two pairs of satellited chromosomes, hybridization would first result in a $2n$ heterozygote with only two satellited chromosomes, allowing for the effect of differential amphiplasty, and doubling would restore the number four in the allotetraploid. This condition is encountered in the *S. alba* and *S. fragilis* complements. Conceivably either one or other of the hypothetical ancestral forms might have had only a single satellited pair. In the first case one satellited chromosome would be present in the transitional heterozygote, if the haploid complement with only one satellite caused the disappearance of both satellites in the other haploid complement; in the second case, only one satellited chromosome would result, whichever parental complement

happened to dominate with regard to differential amphiplasty. Subsequent doubling would produce an equal satellited pair in the allotetraploid, and this is precisely the case in *S. alba* var. *caerulea*. Differential amphiplasty does not, of course, offer the only possible explanation of the halved satellite number in the cricket bat willow. The modification of chromosome structure manifesting itself in a loss of satellite may be related in some way to the environmental factors incidental to the establishment of the variety; in any case such factors must have been indirectly effective through their probable role in the origin of the variation of one of the ancestral diploids.

Though the result of this investigation may not be of immediate practical value, it is not without theoretical interest. The distinction of the bat willow from other *S. alba* varieties on the basis of satellite number is a matter of minute detail, and the observer is not yet proof against the vagaries of fixation and staining; so that the grower of bat willows would as yet be ill advised to rely solely upon the presence or absence of satellites in checking his stock by cytological examination. As has been shown, evidence of hybridity can be obtained if chromosome measurement is attempted, when a bat willow stock is suspected of contamination by *S. fragilis*. This entails a great deal of labour, and up to the present results are only known for the F_1 generation.

But most serious of all, it seems fairly certain on cytological grounds that there are variant strains of *caerulea*, one of the Long Ashton types having been already shown to differ from the other in possessing four secondarily constricted chromosomes instead of two. Turning to other members of the group, the variety *Basfordiana* of *S. fragilis* has a somatic complement indistinguishable from the *alba* karyotype; and one of the 'unknowns' appears to have an *alba* complement except for the suspected presence of four instead of two secondarily constricted chromosomes.

The existence of strains within var. *caerulea* has been noted on more general grounds. Thus Pratt (1934) reiterates an opinion expressed by Burtt Davy that the Chelmer Valley strain is one which growers should be advised to plant; and the Essex growers believe that there is a strain of *S. caerulea* which is more susceptible than others to 'butterfly marking', a swelling of cambial origin which results in a horizontal cracking of the bark, and ruins the clefts.

It is therefore quite certain that, until tests have been very much more extensively carried out, the cytological method should be used at the most as a check on conclusions reached by other means.

Summary

1. A cytological investigation has been undertaken into the constitution of *Salix alba*, *S. fragilis*, and some of their varieties and hybrids, special attention being directed towards *S. alba* var. *caerulea*, the cricket bat willow.

2. *S. alba* and *S. fragilis* are allotetraploids, with seventy-six chromosomes.

3. The somatic chromosomes of these fall into fairly well marked size classes. In the *S. alba* complement the two longest chromosomes have secondary constrictions, affording a distinction between *S. alba* and *S. fragilis*.

4. Two pairs of short, satellited chromosomes are present in both the *S. alba* and *S. fragilis* complements, one pair with and the other without median constrictions.

5. *S. alba* var. *caerulea* differs from *S. alba* in having but one pair of satellited chromosomes with median constrictions.

6. In hybrids between *S. alba* and *S. fragilis*, regular amphiplasty occurs; (*a*) differential amphiplasty which results in a loss of two satellites, and (*b*) neutral amphiplasty, which is revealed in a shortening of some of the chromosomes.

7. *S. caerulea* is considered to be a variety, not a hybrid.

8. The fact that strains exist within *S. caerulea* is correlated with the existence of types with abnormal somatic chromosome complements.

9. For this reason it is not recommended that the cytological test be relied upon in the diagnosis of bat willow sets, but only as a verification of contamination by other species, e.g. *S. fragilis*.

ACKNOWLEDGEMENTS

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Westiellopsis prolifica, gen. et sp. nov., a New Member of the Stigonemataceae [1]

BY

MERCIA JANET

With two Figures in the Text

THIS alga was found, together with *Chlorococcum humicolum* (Naeg.) Rabenh. and other forms, in a culture of soil from the gardens of the Agri-horticultural Society, Madras; the culture medium used was Moore's solution (Moore and Karrer, 1919, p. 285). The alga first appeared as a bluish-green patch on the side of the bottle, just above the level of the culture solution, and later also within the latter.

The branched filaments are devoid of a sheath and are not enveloped in mucilage. The thallus (Fig. 1, *a*) consists of (i) a primary creeping and horizontal portion, with more or less torulose filaments made up of short barrel-shaped cells which are about as long as broad (8–12 μ broad and 6·5–16 μ long), and (ii) a projecting portion consisting of more or less upright branches, arising from the horizontal system and not constricted at the cross-walls, the cells being cylindrical and about twice as long as broad (8–16 μ long and 4–6 μ broad). The cell contents are bright blue-green and slightly granular. The intercalary heterocysts (Fig. 1, *j*) are either quadrate or more often oblong-cylindrical, with light bluish-green contents; they measure 5·5–6 μ in breadth and 10·5–22 μ in length.

In the development of an erect thread a cell of the horizontal portion becomes protruded (Fig. 1, *l*) and the protrusion is cut off by a horizontal wall (Fig. 1, *b*), the upper cell dividing to form the branch. This alga thus shows true branching. The branches usually arise at some distance from one another (Fig. 1, *a*), although they may arise in large numbers close together (Fig. 1, *k*). The apical portions of the erect filaments widen and become more torulose (Fig. 1, *d*) and at first resemble the early stages in the formation of hormocysts in *Westiella intricata* as figured by Borzi (1917, pl. 9, Fig. 43). Especially in older plants lateral branches may arise from some of the rounded cells (Fig. 2, *f*).

These hormocyst-like terminations are not, however, enclosed in any sheath as is the case in the hormocysts of Westiella or Leptopogon (Geitler, 1932, Figs. 337–9 and 344), but simply consist of a single row of enlarging and

rounded cells (Fig. 1, *d*). Later, these cells divide both transversely and longitudinally (Figs. 1, *c, e*; 2, *a*) and ultimately give rise to irregular clusters of rounded cells (Fig. 2, *b, c*), the contents of which escape as rounded gonidia
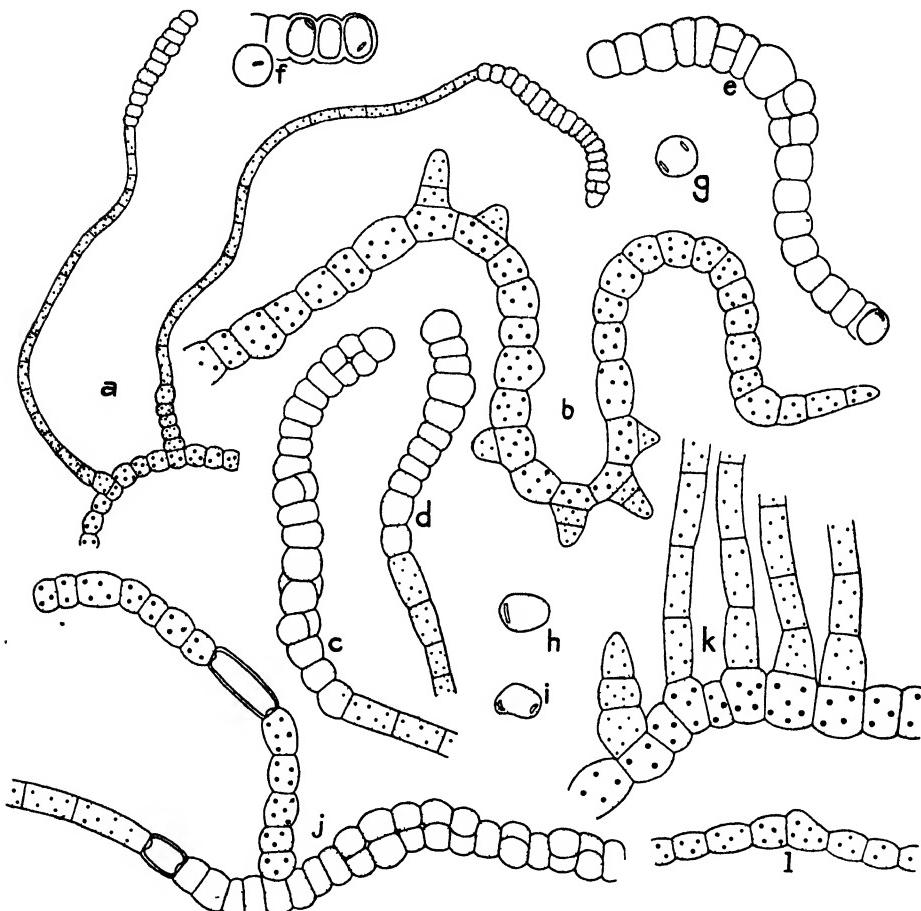
FIG. 1. *Westiellopsis prolifica*, nov. gen et sp.: *a*, mature plant; *b*, filament showing branch-formation; *c, d*, young pseudohormocysts, in *c* showing longitudinal division; *e*, ditto, showing escape of gonidium; *f*, gonidia with refractive bodies; *g–i*, free gonidia with refractive bodies; *j*, filaments with heterocysts, a branch and a pseudohormocyst; *k*, prostrate filament with plentiful branching; *l*, early stage of branch-formation. (*a* × 140; the rest × 300.)

through rupture of the cell-membrane (Figs. 1, *e, f*; 2, *c*); only one gonidium is formed from each cell. Plenty of escaped gonidia are found lying freely in the water. The gonidia (Fig. 1, *f, h*) are 8·5–9 μ in diameter, possess a delicate cell-wall, and have homogeneous, pale bluish-green contents. They enclose a peculiar refractive body situated towards one side of the cell, or sometimes two such refractive bodies are present, one at each end (Fig. 1, *g, i*). These

bodies are present in the gonidia already before their escape (Fig. 1, *f*). Germination of the gonidia commences soon after liberation. A protrusion is formed on one side, which is then cut off by a transverse wall (Figs. 1, *i*; 2, *d*), and further division soon results in a filamentous structure (Fig. 2, *e*).

The method of branching shows that this alga belongs to the Stigonema-
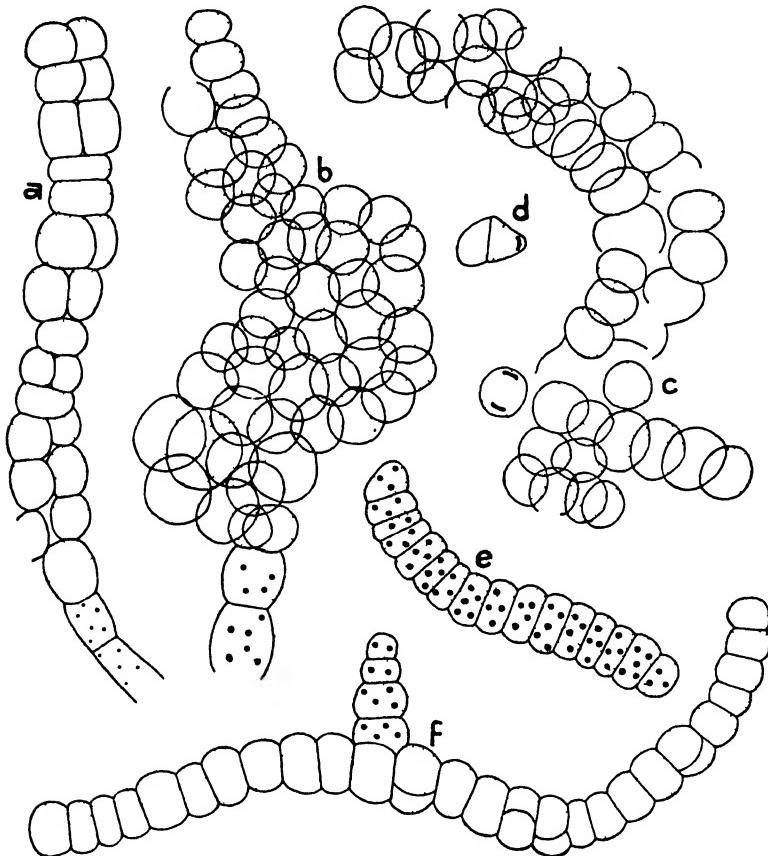
FIG. 2. *Westiellopsis prolifica*, nov. gen. et sp.: *a*, pseudohormocyst showing longitudinal division and one cell from which a gonidium has been liberated; *b*, the same forming clusters of rounded cells; *c*, the same showing escape of gonidia; *d*, germinating gonidium; *e*, young filament produced from same; *f*, branch-formation on the pseudohormocyst. (*b, c* × 560; the rest × 430.)

taceae. It resembles some species of Stigonema in the possession of a single row of cells and in having prostrate and erect filaments. In the slight difference between the two it approaches Fischerella, but it differs from both these genera in having a specialized structure at the ends of the filaments, in which respect there is a resemblance to the hormocysts of Westiella and Leptopogon. When the hormocyst-like apices are young, there is also a certain amount of similarity to Chondrogloea (Geitler, 1932, p. 550), where the lateral branches

are narrow and cylindrical at the base, but torulose above, although again becoming cylindrical towards the tip. Gonidium-formation has, however, never been reported from the torulose intercalary portions of Chondrogloea.

The dilated apices of the branches of this alga resemble the hormocysts of Westiella and Leptopogon in their position and general appearance and in the detachment of portions which grow into new plants. But they differ in the fact that they lack a sheath, that they undergo both transverse and longitudinal division, forming two or more longitudinal rows of cells, and especially that formation of gonidia takes place from the cells. They therefore do not agree with the customary definition of a hormocyst (Geitler, 1932, p. 37) except in their terminal position and their frequent detachment. from the filament. They are best regarded as hitherto undescribed structures which may be called 'pseudohormocysts', and in view of their presence the alga is best referred to a new genus, Westiellopsis, which may be placed in the Stigonemataceae next to Chondrogloea.

Westiellopsis gen. nov.

Thallus filamentous with true branching; filaments of two kinds, primary filaments slightly thicker and more or less creeping, secondary filaments, thinner and generally growing erect; filaments without a sheath and consisting of one row of cells; heterocysts intercalary; the dilated terminal portions of the secondary branches, by profuse transverse and longitudinal division, form clusters of rounded cells (pseudohormocysts) the contents of which escape as ·gonidia and develop into new plants.

Westiellopsis prolifica sp. nov.

Characters same as for the genus; main filaments torulose and consisting of short barrel-shaped cells, 8–12 μ broad and as long as broad or slightly longer; branch-filaments thinner and elongate, not constricted at the cross-walls, with elongate cylindrical cells, 4–6 μ broad; heterocysts oblong-cylindrical, 5·5–6 μ broad and 10·5–22 μ long. Gonidia formed singly from each cell of the pseudohormocyst, 8·5–9 μ in diameter.

In cultures of soil algae from the Agri-horticultural Society's Gardens, Madras.

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BORZI, A., 1917: Studi sulle Mixoficee. II. Stigonemaceae. Nuov. Giorn. Bot. Ital., N.S. xxiv.

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NOTE

THE EFFECT ON THE FORMATION OF CARBOHYDRATES IN LEAVES OF THE OMISSION OF RED AND BLUE-VIOLET RAYS FROM ELECTRIC LIGHT.

—Dastur and his collaborators (Dastur, R. H., and Samant, K. M., Ann. Bot., xlvii. 295, 1933; Ind. Journ. Agr. Sci., iii. 460–77, 1933; Dastur, R. H., and Mehta, R. J., Ann. Bot., xlix. 808–22, 1935, and Dastur, R. H., and Solomon, S. S., Ann. Bot., N.S. i. 147–52, 1937) concluded that both red and blue-violet light are important for the normal photosynthetic activity, in that with white light the carbohydrate balance depends on the distribution of radiation of different wave-lengths. It is also established that in red (6,200–7,000 Å.) light the formation of carbohydrates in the leaves is less than white light of the same energy value, while it is higher than in blue-violet light (4,000–4,700 Å.) alone. It would seem, therefore, of interest to study the effect on the formation of carbohydrates in leaves of the omission from ordinary light of the red rays and of the blue-violet rays.

Preparation of the filters. For absorbing red rays a solution of pure cupric nitrate ($Cu(NO_3)_2\ 3H_2O$) in distilled water was prepared, and it was found that 667·6 gm. in 1,000 c.c. gave the violet-orange range. Similarly 33·4 gm. of chromic acid in 1,000 c.c. gave the green-red range, absorbing the blue-violet rays.

With each solution the transmission was determined with a photo-electric cell, and it was found that with electric light (daylight bulb) the transmission of the cupric nitrate was 44 per cent., while that of the chromic acid solution was 98 per cent. The range of transmission (as determined by the spectro-photometer) of the first was 3,970–6,010 Å. and of the second 5,150–7,880 Å.

It was not possible to use a thermopile to determine the intensity of light when the copper solution was used. An indirect method was accordingly employed. The intensity of light received at a distance of 10–12 in., passing through the chromic acid filter, as measured by the thermopile, was 7·7 cm. on the galvanometer scale when the electric lamp (daylight bulb) was about 50 cm. above the cooler. The cupric nitrate solution with 44 per cent. transmission should give a deflection of 3·5 cm. approximately. Hence the intensity received at a distance of 10–12 in. below the chromic acid filter had to be reduced to a deflection of 3·5 cm. This was achieved by using glass plates as employed by previous workers. Similarly, the intensity of ordinary light was reduced by the use of three glass plates. Thus the batches of plants in the three different lights were exposed to nearly equal intensities in each case. Care was taken that the temperature surrounding the plants was the same as the room temperature.

The time of exposure and the methods for the analysis of carbohydrates were the same as used by previous investigators in this laboratory (Dastur et al., loc. cit.).

Results. The results of carbohydrate analysis of the leaves of *Helianthus annuus*, L., *Abutilon asiaticum*, G. Don., and *Tropaeolum majus*, L., exposed to lights of various ranges as given in the preceding pages are given in Table I.

TABLE I

Total Carbohydrate Content of the Leaves before and after Exposure to Different Lights
(gm. per 100 gm. of fresh leaves)

Intensity of light in each case = 3·5 cm. deflection.

Experiment.	Dark.	Ordinary electric light (3,970–7,880 Å.)	Blue-violet rays absorbed (5,150–7,880 Å.)	Red rays absorbed (3,970–6,010 Å.)
		Helianthus annuus		
1	0·0286	0·0617	0·0567	0·0545
2	0·0155	0·0331	0·0249	0·0225
3	0·1150	0·1213	0·0713	0·0587
4	0·0675	0·0589	0·0558	0·0415
5	0·0255	0·0738	0·0447	0·0320
6	0·0438	0·0808	0·0571	0·0549
Mean	0·0493	0·0716	0·0517	0·0440
		Abutilon asiaticum		
1	0·0478	0·2558	0·2304	0·1721
2	0·0492	0·1170	0·0748	0·0619
3	0·0398	0·0674	0·0315	0·0251
4	0·0440	0·0999	0·0657	0·0547
5	0·2015	0·1451	0·1442	0·1287
6	0·0442	0·0846	0·0811	0·0731
Mean	0·0713	0·1283	0·1049	0·0859
		Tropaeolum majus		
1	0·0387	0·0826	0·0658	0·0596
2	0·0471	0·1387	0·1202	0·0519
3	0·0650	0·3810	0·2132	0·1113
4	0·1037	0·1203	0·0757	0·0689
5	0·0591	0·1975	0·1448	0·0909
6	0·0635	0·1485	0·1106	0·0887
7	0·0664	0·2605	0·1745	0·1221
Mean	0·0634	0·1899	0·1292	0·0849

It was necessary to test the significance of the differences in the total carbo-hydrates formed in the three illuminations by employing Fisher's method of '*t*'. Table II shows that the differences found are statistically significant.

TABLE II

	Helianthus annuus $n = 5$.	Abutilon asiaticum $n = 5$.	Tropaeolum majus $n = 6$.
Ordinary light vs. light minus the blue-violet	$t = 2.679$ $P = 0.05$	$t = 3.304$ $P = 0.05$	$t = 3.039$ $P = 0.05$
Ordinary light vs. light minus the red	$t = 3.197$ $P = 0.05$	$t = 3.918$ $P = 0.02$	$t = 3.395$ $P = 0.02$
Light minus the red vs. light minus the blue-violet	$t = 3.154$ $P = 0.05$	$t = 2.187$ $P = 0.10$	$t = 3.355$ $P = 0.02$

From earlier results it was not clear whether the remaining portion of the visible spectrum, the yellow-green region, had any role to play in the process. The results obtained in this investigation throw some light on the point. It appears that the

yellow-green region is very slightly effective in the absence of the red rays, as the omission of the red rays from the visible spectrum has resulted in very low assimilation in the leaves; and it is likely that such photosynthetic activity as was observed was due to the blue-violet rays in the light from which the red had been absorbed, since a feeble photosynthetic activity was also obtained in the blue-violet light by previous workers.

Conclusion. The results show that the quantities of carbohydrates formed in ordinary electric light are higher than the quantities formed when either the blue-violet rays or the red rays are removed by means of suitable filters, the total energy value of the light being kept the same. The depression is greater with the absorption of the red rays than with that of the blue-violet. Carbohydrate formation is however greater in white light than in the light after the loss of the blue-violet rays, although such light contains a higher proportion of red rays than the white light.

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The Growth of Isolated Cotyledons of Cucurbita Pepo

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With four Figures in Text

INTRODUCTION

IT has long been known that cotyledons isolated from the remainder of the seedling show capacity for growth and regeneration. At the present attention is particularly directed to the possible part played by hormones in development and regeneration phenomena, somewhat to the exclusion of the study of other factors. In many cases the effects of growth-promoting substances are assessed by such simple criteria as extension growth or turgor changes, so that in the final analysis the effects of the specific growth substances are manifested through changes in water relations: yet it is not these latter processes and their control by the auxins which chiefly receive attention. Study is rather concentrated on the more striking regenerative changes which in many cases accompany them.

The culture of isolated cotyledons was first studied by Sachs (1859) using *Phaseolus vulgaris*. He was concerned with the change in capacity for regeneration at various times after germination. The formation of chlorophyll and roots has been observed by Blociszewski (1876) in *Pisum sativum* and *Lupinus luteus*, Küster (1903) in various Cucurbits, Nakano (1924) in *Vicia faba* and *Phaseolus vulgaris*, Kanzler (1925) in *Galium Aparine*, Kowalewska (1927) in *Phaseolus multiflorus*, *P. vulgaris*, and *Pisum sativum*, Fuja (1929) in Cucurbita, Cucumis, and Lupinus, by La Rue (1933) who cultured excised cotyledons of some seventy-three species of flowering plants, and by Wilson (1933) in *Medicago sativa*. The formation of shoots was not always noted. Küster (1903) found them to occur sporadically, Kowalewska (1927) states that shoots form only after three weeks as compared with three days for roots. Fuja (1928) distinguished two types of shoot regeneration, from axillary buds of the cotyledon, and from adventitious buds from wound callus. La Rue (1933) found shoot formation in many of the species studied. In *Cucurbita Pepo* roots formed in nine and shoots in seventeen days, and as precautions were taken to remove axillary buds these shoots were adventitious. No shoots were obtained by Blociszewski (1876), Nakano (1924), or Wilson (1939).

These investigations were of a purely qualitative nature. Thus it was established that fragments of cotyledons would regenerate (Blociszewski)

more actively if taken from basal than apical parts of cotyledons (Fuja), and also as readily in the dark as in the light (La Rue). In the present investigation these observations have been confirmed, but the main attention has been directed to a quantitative study of the growth of the isolated cotyledons, and the effect of the external factors concerned. It had been hoped to extend the study to the hormone relations, but the work has had to be interrupted. Incomplete as the work remains, it seems justifiable to put on record the results obtained.

MATERIALS AND METHODS

The extension of the investigation so as to obtain quantitative results involves considerable modification in the technique of culturing. In a preliminary series of experiments isolated cotyledons of Cucurbita Pepo were cultured on moist sand and filter-paper, and these have been generally used. On such media the cotyledons increased in size, turned green and developed roots, but development, even in the same dish, was extremely irregular. This was due apparently to three causes: (1) there are within any sample of commercial seed very large variations in both size and form, which it would seem affect the rate of growth very considerably; (2) unless aseptic conditions are used the cotyledon is very susceptible to bacterial attack, and then either succumbs or grows irregularly; (3) small variations in water-supply seriously affect development. In some of the Petri dishes in which cotyledons were grown it was noticed, for example, that those at the edge turned green earlier than did those at the centre, and this was attributed to the effect of a furrow at the edge of the dish into which water could drain from the centre.

The variability of the sample of seed used can of course be diminished by selection to conform with certain standards of weight and shape, and only those weighing 180–220 mgm. were employed. The sample thus obtained was further selected by eye for shape. All thick or abnormally thin seeds were rejected; only those of a standard intermediate shape were used.

Aseptic methods were employed by some earlier workers. Nakano (1924) washed the grains in sterile distilled water, separated the cotyledons from the rest of the seed aseptically, and cultured them on sterile media. Kowalewska (1927) used sterile media but not aseptic methods in the preparation of the culture. La Rue (1933) sterilized the seeds, from which he afterwards removed the cotyledons, in Wilson's (1909) solution of calcium hypochlorite; they were transferred aseptically and cultured on sterile media. Calcium hypochlorite was also used for sterilization in this investigation. The seeds were soaked in this solution for three hours, and effective sterilization of the surface of the outer seed-coat membrane was thus secured without apparently affecting the cotyledons or embryo. Only after the seed has been in the solution for more than three hours does seepage through the micropyle begin. The seeds were then removed to sterile distilled water.

The procedure followed in the removal of the cotyledons from the seed and their separation from the inner seed-coat membrane has been described elsewhere (Brown, 1940). The purpose in this work, however, was the extraction of the cotyledon and not, as in the earlier investigation, the inner seed-coat membrane, which necessitated an elaboration of the method to ensure sterile conditions. The manipulations were performed between two glass plates which were swabbed at frequent intervals with alcohol, and conditions were maintained as nearly sterile as possible. The whole process occupied two to three minutes and, in spite of the precautions taken, the proportion of infected cultures always reached about 20 per cent. Needless to say in the selection of the sample for measurement these were rejected.

The high rate of infection made it necessary to adopt measures for its localization when it occurred. The cotyledons were therefore cultured either singly or in pairs in Petri dishes 1·5 in. in diameter, which readily accommodated the two cotyledons even after fourteen days' growth.

Irrespective of the medium cotyledons do not grow successfully when arranged vertically with their basal ends thrust into the supporting material. Uniform and rapid development occurs only when they are cultured flat upon the medium. This imposes two conditions which must be met: the surface on the medium must be supplied with free water, but, at the same time, preserve adequate means for free gaseous exchange.

All the results presented here were obtained with cotyledons grown on very small glass beads to which the requisite amount of water had been added. A high and uniform level of water-supply on this medium is possible without preventing the access of oxygen to the surface in contact. Enough water was added to fill all the interstices of the lower, but not those of the upper three or four layers of beads; the cotyledons were then in contact with the free water in capillary films over the beads in the upper layers, and free exchange of gases occurred through the interstices. The level of water availability could be reduced by reducing the amount of water added to a given bulk of beads.

The necessity of maintaining sterility precluded the possibility of making serial observations on the same sample of cotyledons; these could only be obtained from random samples withdrawn from a large population. In spite of the measures adopted to reduce variability this was fairly high, as indicated by the statistical data presented in the next section; the sample therefore had to be correspondingly large. These experiments were designed to provide samples of ten dishes, allowance being made for a 24 per cent. mortality due to infection. On each of the samples measurements of one or more of the following characteristics were made: (1) fresh weight, (2) dry weight, and (3) surface area. For the determination of fresh weight the cotyledons, after removal from the Petri dishes, were pressed between several layers of filter-paper to remove surface moisture and then weighed. The same material after drying in an oven for about twelve hours at 95° C. was weighed a second time.

The estimation of the change in dry weight with time could not, however,

be accurately made by a comparison of the successive sample-means. The change is very small and is usually swamped completely by the sampling error. It could only be measured by relating the dry weight of each cotyledon to its dry weight before the experiment began. Were it not for the necessity of maintaining sterility this could have been done by weighing the individual cotyledons after extraction from the seed, and by estimating their dry weight from the mean water content of a large sample; a method which gives dry weight values to within 1 mg. After separation from the seed these cotyledons had to be transferred immediately to the Petri dishes, and their fresh weight could therefore be estimated only from the difference between the weight of the entire seed and that of the discarded portions. A further complication is introduced by the fact that the water content of the discarded fragment is raised by the soaking in calcium hypochlorite. Before weighing this was reduced to the original level by drying the discarded portions over strong sulphuric acid and under a reduced pressure for about 18 hours.

In some experiments measurements were made of the change in area. After removal from the Petri dish, and before the fresh weight was determined, a tracing of the outline of the cotyledon was made, the area of the figure being subsequently measured with a planimeter.

The experiments were all conducted in a chamber at 25° C. The cultures were illuminated by two 1,000-watt lamps provided with a screen of running water. At the level of the table on which the dishes were placed the light intensity was about 200 foot-candles. In these experiments the effects of this intensity were contrasted with those of 50 f.c., obtained by covering the cultures with two layers of tissue-paper.

In addition to light intensity and water content, the effect of two other factors has been examined: (1) contact with the substrate of the inner and outer surfaces respectively, (2) the presence or absence of the inner seed-coat membrane.

In this paper the two surfaces are characterized as 'inner' and 'outer' according to the position each occupies in the seed. The inner flat surface in the seed is pressed against the other member of the pair of cotyledons; the outer rounded surface is normally covered by the inner seed-coat membrane which, in the dry state, adheres firmly. After the emergence of the seedling from the seed-coat the inner surface becomes the upper surface of the green cotyledon. In the last two sections of this paper the inner seed-coat membrane is referred to as the 'pellicle'.

<div align="center">EXPERIMENTAL RESULTS</div>

(a) *General observations.* The cotyledons cultured by the methods described above differed considerably from those of normally developed seedlings; they developed more rapidly and they had a highly characteristic appearance. In Table I the fresh weights of isolated and attached cotyledons are compared during the first six days of development. The values show the effect of the

removal of the pellicle on the development of each set. The seedlings from which the cotyledons for this experiment were taken were grown on beads carrying the same amount of water as those on which the isolated organs were cultured; and the two series were exposed to the same light intensity of 200 f.c. It is evident that as between comparable groups of isolated and attached organs the greatest development is always shown by the former. The removal of the pellicle accelerates development in both cases, but even where the comparison is made between isolated cotyledons with pellicles and attached cotyledons without pellicles, the latter only overtake the former at about the sixth day.

TABLE I

Mean Fresh Weights (mg.) of A, attached, and I, isolated, cotyledons.
P+ indicates pellicles remaining; P— indicates pellicles removed

Days.	I P—	I P+	A P—	A P+
2	110·5	105·1	50·5	52·0
4	174·6	165·8	75·7	71·2
6	261·3	205·3	224·4	96·1

In addition to the fresh weight differences the qualitative differences between isolated and attached cotyledons are also great. Cotyledons cultured in isolation when mature are broader, becoming almost circular in outline; they are thicker, more brittle, and in a light intensity of 200 f.c. they develop a much darker green colour. The most striking difference, however, is due to an inrolling of the edges towards the original outer surface, which may occur from the sides or from the base and apex of the cotyledon. This last characteristic may be due to abnormal divisions over the inner surface. In the resting cotyledons there is a well-marked palisade below the epidermis of this surface, but as a result of frequent anticlinal divisions this tissue becomes disorganized.

As other workers have found, the cotyledons in culture turned green and formed roots. La Rue's statement that root formation occurs as vigorously in the dark as it does in the light was confirmed. But whereas this worker observed the frequent formation of adventitious buds, this was only noticed twice in the present series of experiments; the two cases, however, were very similar to those described by La Rue. The two cotyledons had apparently been severed from the seed together with a fragment of the embryonic tissue of the petiole, which extended and from its upper surface, about half-way along its length, there developed a shoot.

Some of the cotyledons that had formed roots in the Petri dishes were transferred to sand and watered with a nutrient solution. They increased rapidly in size and at the end of three weeks were larger than the cotyledons of a normal seedling. A similar observation was reported by Blociszewski (1876).

(*b*) *Quantitative results*. The quantitative changes which occur in the

course of development were investigated in four experiments in which the fresh weight, dry weight, and surface area were measured at intervals of 24 hours. All four experiments had the same cultural conditions, a fairly high
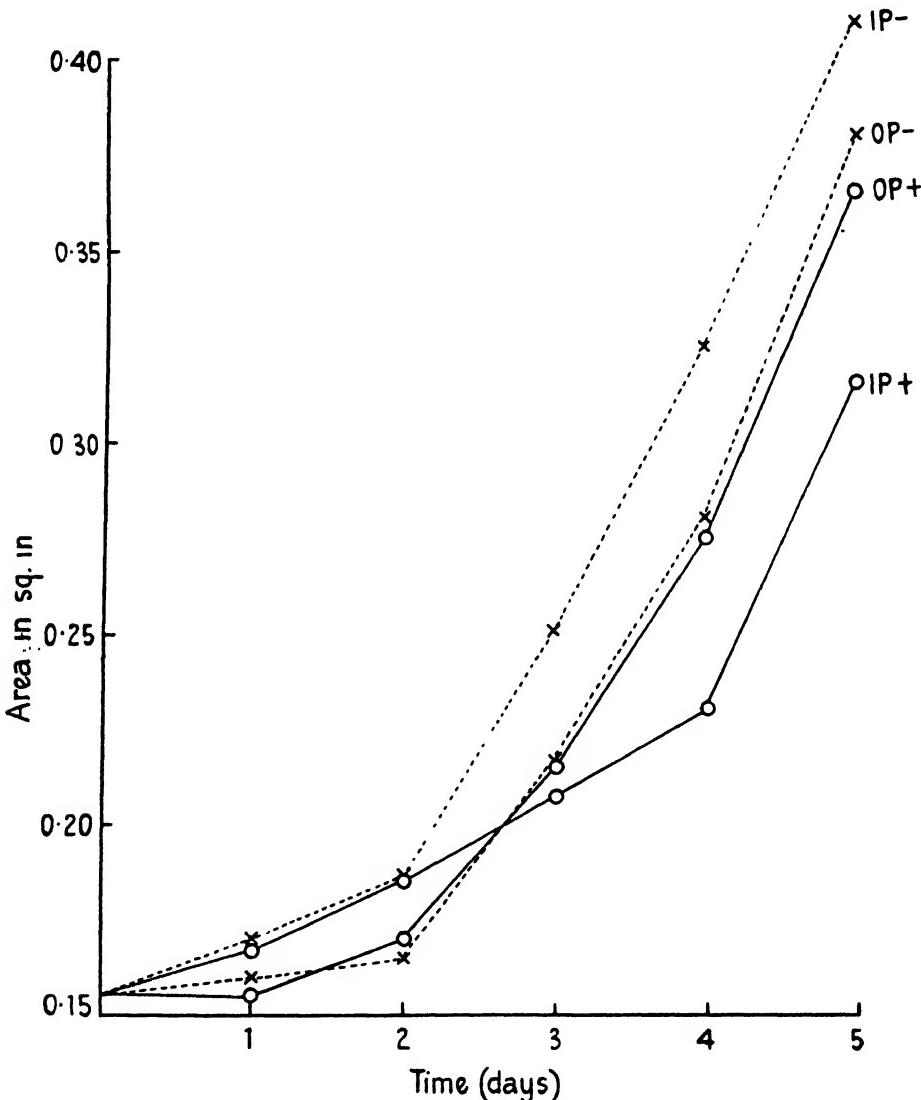
FIG. 1. Curves of the growth in area of isolated cotyledons. I, inner surface in contact with substrate; O, outer surface in contact with substrate; P+ and P—, presence and absence of pellicle.

level of water-supply and light intensity of 200 f.c. The factors, presence and absence of pellicle, and contact on the substrate with upper and lower surfaces respectively, were combined to give four series as follows: (1) inner surface in contact and pellicle removed, (2) outer surface in contact and pellicle removed,

(3) outer surface in contact and pellicle attached, (4) inner surface in contact and pellicle attached. The data of these four experiments are presented graphically in Figs. 1 and 2, and in Table III.

The curves of Fig. 1 show the changes in area with time in the four series.
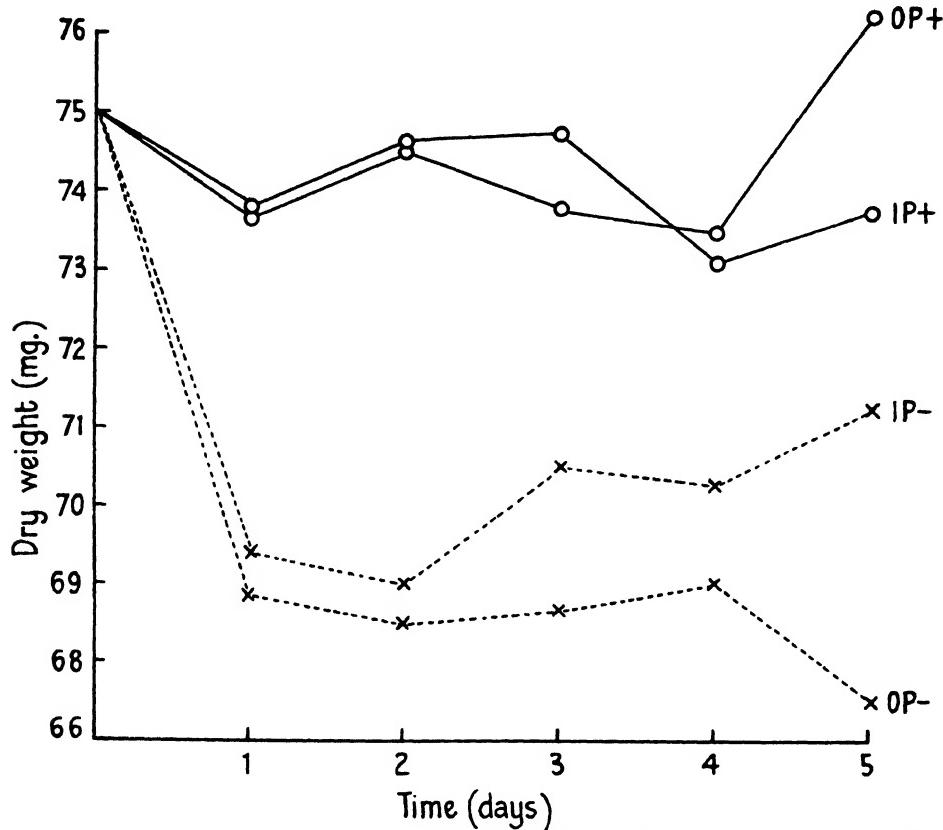
FIG. 2. Dry weight of isolated cotyledons. I, inner surface in contact with substrate; O, outer surface in contact with substrate; P+ and P−, presence and absence of pellicle.

Table II presents a summary of the data of the statistical analysis of the area measurement of the final samples in this experiment.

The effects of pellicle removal and of interaction are statistically significant, that of the particular surface in contact is not. Since it is only the effect of pellicle removal that is independently significant, the interaction must be due to a limitation imposed on the effect of pellicle removal by the surface in contact. The difference between the means for pellicle present and absent when the outer surface is in contact are not significant, but it is so when the inner surface is in contact; it would therefore seem that it is only under these conditions that pellicle removal has any effect.

The mean water contents as percentage of fresh weight for the same series

TABLE II

Summary of Statistical Analysis of Individual Values of Final Samples of Four Experiments: contrasting Effects of P+ Presence and P— Absence of the Pellicle, and of Contact with the Substrate of I Inner and O Outer Surfaces

	I P—	O P—	O P+	I P+
Mean area (sq. in.) . .	0·405	0·384	0·359	0·315

Analysis of variance:

	D.F.	Sum. of dev. sq.	Variance
O v I	1	0·21	0·21
P— v P+ . . .	1	3·13	3·13
Interaction . . .	1	1·13	1·13
Error	28	9·46	0·332

S.E. of diff. between two means = 0·024.

TABLE III

Mean Water Content (% of fresh wt.) of Cotyledons; with P+ Pellicles, P— without pellicles; Contact with the Substrate of I Inner and O Outer Surfaces. All exposed to light of 200 f.c. High water availability

Hours.	O P—	O P+	I P—	I P+
0	8·2	8·2	8·2	8·2
24	30·6	30·8	32·0	33·5
48	37·9	36·0	38·1	37·3
72	48·2	47·5	50·1	47·0
96	57·5	57·2	64·4	55·0
120	64·6	—	71·7	—

are presented in Table III. The following results may be noted. The water content with outer surfaces in contact finally lies between the values for the inner surface in contact. Removal of the pellicle had finally no effect when the outer surface is in contact, but a large effect when the inner surface is supplied with water. These results therefore resemble those already found for area of cotyledons. A very rapid increase in all series occurs during the first 24 hours, followed by a reduction in the rate of water uptake during the second day. After this a renewed rapid uptake occurs in all series. During the initial phase the two series with the inner surface in contact show more rapid uptake, and the effect of removal of the pellicle is in this phase very small.

Dry weight changes in the four series are shown in Fig. 2. The outstanding effect is that due to the removal of the pellicle, for irrespective of the surface in contact with water a very rapid reduction in dry weight occurs in the absence of the pellicle but is largely prevented by its presence. Subsequent to the first day little change in dry weight occurs in any series.

A series of experiments was also undertaken to investigate the effect of light intensity on the growth of the cotyledon as determined by the water content. The first of these results which are shown in Table IV concerns the effect of difference in light intensity when the upper and the lower surface were supplied with restricted water-supply. In this experiment control by

the pellicle was eliminated by its removal. The light intensities used were 200 and 50 f.c.

TABLE IV

Mean Water Content (% of dry wt.) of Cotyledons without Pellicles. I, Inner Surface in contact with Water; O, Outer Surface in contact with Water. L, exposed to 50 f.c., M, exposed to 200 f.c. Low water availability

Hours.	O L	O M	I L	I M
0	8·9	8·9	8·9	8·9
24	41·0	42·2	50·1	53·2
48	44·8	53·7	54·6	64·6
72	104·9	80·6	106·0	114·3
96	129·5	93·4	118·7	120·6
120	168·8	103·6	135·6	144·0

In Table V is presented a summary of the statistical analysis of the combined data of the last two samples of the experiment of Table IV.

TABLE V

Summary of Statistical Analysis of combined values of last two Samples of the experiment in Table IV. Symbols as in Table IV

	I M	O M	I L	O L
Mean water content . .	132	98	127	148
Analysis of variance:				
	D.F.	Sum. of dev. sq.	Variance.	
M v L	1	5522·4	5522·4	
I v O	1	10325·9	10325·9	
Interaction . . .	1	7340·0	7340·0	
Error	36	81838·0	887·0	

S.E. of diff. between two means = 13·1.

The effects of light intensity, surface exposed, and of interaction are all significant. The interaction in this case is clearly due to the restriction imposed by each factor on the independent action of the other. High light intensity depresses growth, but only when the outer surface is in contact with the medium and the inner surface therefore exposed to light. The difference between inner and outer surfaces in contact is statistically significant only when the light intensity is high. The series I M and O M in this experiment correspond with the series O P— and I P— in Fig. 1 and Table II. In the latter the highest growth is made by cotyledons with the inner surface in contact, but the difference between these and the series with the outer surface in contact is not statistically significant. The general difference, however, agrees with that given by the results of Table V.

Figs. 3 and 4 represent graphically the results of experiments with the same two light intensities, in the presence and absence of the pellicles. In these experiments all the cotyledons were arranged with their inner surfaces in contact with the medium. The water-supply was also varied, being medium in Fig. 3 and high in Fig. 4. To assess the effect of low water-supply the corresponding values in Table IV (series I L and I M) may be compared.
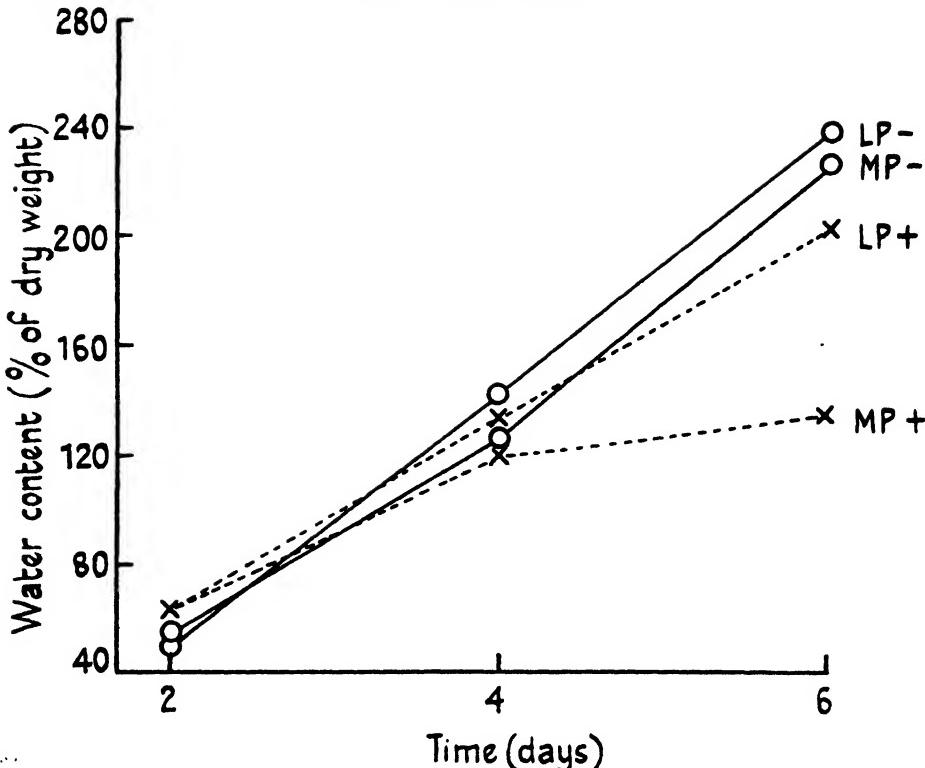
FIG. 3. Water content of isolated cotyledons. L, light intensity 50 foot-candles; M, light intensity 200 foot-candles; P+ and P—, presence and absence of pellicle. Water-supply of *medium* level in all cases.

It is evident that the level of water-supply is the main factor controlling water content.

A summary of the statistical analysis of the individual values of the final samples of Figs. 3 and 4 is presented in Tables VI and VII.

TABLE VI

Summary of the Statistical Analysis of Individual Values of Final Samples of Four Series in which the Inner Surface was in contact with the Medium and in which the Variables were two Light Intensities 200 (M) and 50 (L) foot-candles, and the Presence (P+) and the Absence (P—) of the Pellicle. Intermediate water availability

		M P—	L P—	L P+	M P+
Mean water content .	.	227	238	203	132

Analysis of variance:

				D.F.	Sum. of dev. sq.	Variance.
M v L .	.	.	.	1	71561	71561
P— v P+	.	.	.	1	36680	36680
Interaction	.	.	.	1	42013	42013
Error	.	.	.	36	89352	2493

S.E. of diff. between two means = 22·3.
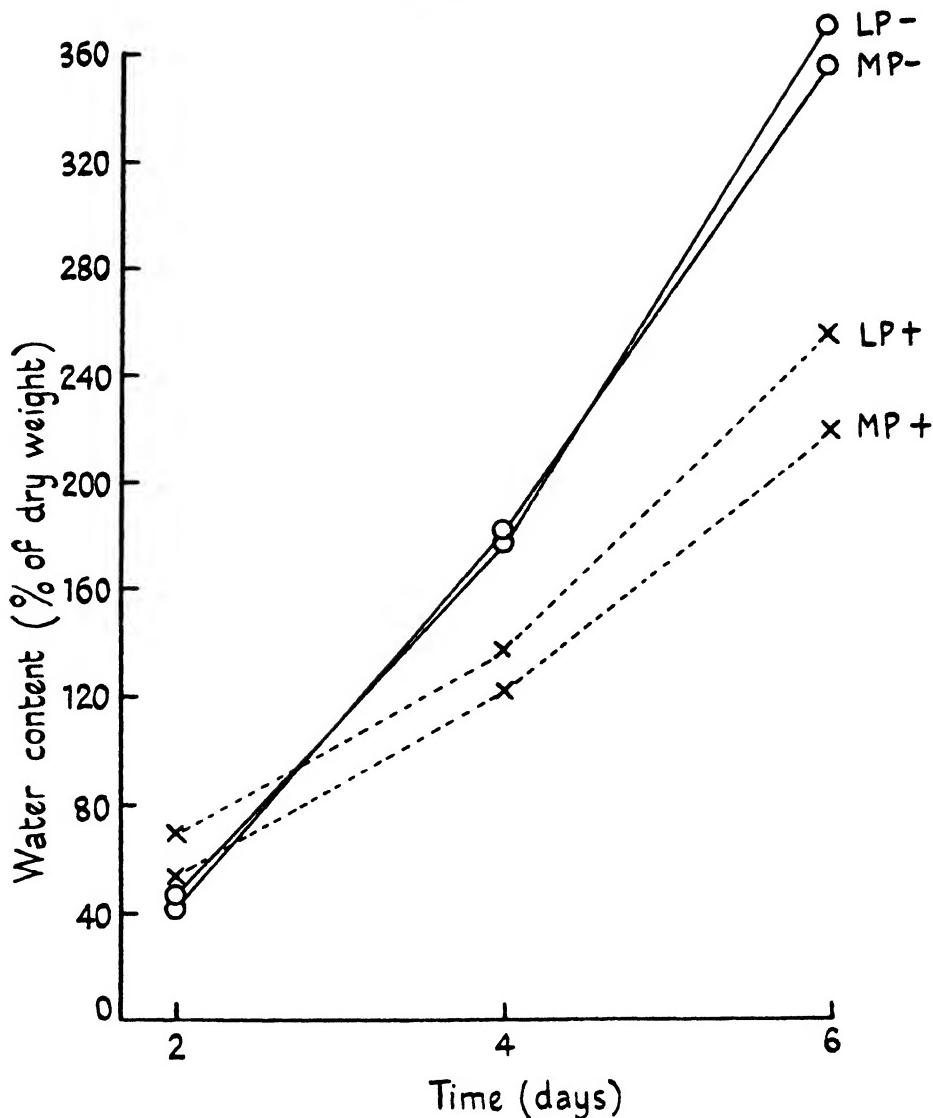
FIG. 4. Water content of isolated cotyledons. The conditions are similar to those of Fig. 3, but the water-supply was of *high* level in all cases.

The light, pellicle, and interaction effects in Table VI are all statistically significant. In this case again the interaction is evidently due to the restriction imposed by each factor on the independent action of the other. The removal of the pellicle leads to the greatest increase in high light intensities; in low light the removal shows a slight increase which is, however, not statistically significant. The influence of light is negligible when the pellicle has been

removed, but considerable when the outer exposed surface is covered by the pellicle.

TABLE VII

Summary of the Statistical Analysis of Individual Values of Final Samples of Four Series in which the Inner Surface was in contact with the Medium, and in which the Variables were two Light Intensities 200 (M) and 50 (L) foot-candles, and the Presence (P+) and the Absence (P—) of the Pellicle. High water availability

	M P+	L P+	M P—	L P—
Mean water content . .	212	259	360	373
Analysis of variance:				

	D.F.	Sum. of dev. sq.	Variance.
M v L	1	9272	9272
P— v P+ . . .	1	65062	65062
Interaction . . .	1	2869	2869
Error	36	40544	1154

S.E. of diff. between two means = 15·2.

In Table VII the light and pellicle effects are statistically significant, but the interaction effect is not. These figures, and the curves of Fig. 4, indicate that in this experiment removal of the pellicle accelerates growth both in low and in high light intensity. The accelerating effect of pellicle removal in high light intensity when the inner surface is in contact with the medium is also shown by the curves of Fig. 1 (series I P— and I P+) and those of Fig. 3 (series M P— and M P+). In Fig. 3 the effect of pellicle removal in low light intensity (L P— and L P+) is also to increase growth; but whereas in Fig. 4 the difference between the series with and the series without pellicle is statistically significant, in Fig. 3 it is not.

Although in Table VII and Fig. 3 there is no interaction between light intensity and the presence or absence of the pellicle there is some indication that the effect of light differs according as the outer exposed surface is naked or is covered by the pellicle. The difference between the light series is not significant with pellicle removed, but it is so with the pellicle attached. This agrees with the result established in the experiments of Table VI and Fig. 3.

The effect of the variables studied in the whole series of experiments may be summarized as follows:

1. The removal of the pellicle increases both the water content and the area of the cotyledon when the inner surface, but not when the outer, is placed in contact with water.

2. Irrespective of the orientation of the cotyledon on the medium, the removal of the pellicle occasions a sharp fall in dry weight.

3. High light intensity decreases the water content when the inner surface is uppermost.

4. The effect of light when the inner surface is in direct contact with water depends upon the presence or absence of the pellicle on the outer surface.

With the pellicle absent light intensity has little or no effect; with the pellicle present high light decreases the water content.

5. The level of water availability in the culture medium affects profoundly the water content and therefore the growth of the cotyledons.

DISCUSSION OF RESULTS

The development of a cotyledon from a colourless dormant organ to an active green structure is characterized by certain changes in the component tissues. During the first 48 hours there are no apparent anatomical changes, for at the end of this period there are still no intercellular spaces, and the cell-walls enclose a continuous mass of cytoplasm, in which oil and protein granules are embedded. After this stage, however, the character of the superficial layers of cell changes. They show cell extension, followed by vacuolation, with the consequent development of intercellular spaces, and at the same time chlorophyll appears. At first these changes are restricted to the surface layers, which enclose a mass of still apparently dormant cells; but with time successively deeper layers are involved until eventually the changes initiated at the surfaces complete the transformation of the whole cotyledon.

It must therefore be the reactions of the surface layers that are of primary interest in the analysis of the effects of different factors in the growth of the entire organ. Evidence has been put forward to show that the reaction of the cotyledon to light differs according to which surface is exposed. The anatomical changes observed in development suggest that the immediate effect of the factors studied is localized in the surface layer, and that the effect on the whole cotyledon is secondary.

Although the change from the dormant to the active state does not occur simultaneously throughout the whole cotyledon, nevertheless the development of activity in the surface layers separates two distinct phases in the development of the cotyledon, these phases being reflected by the water-content values of Table III. The change that occurs at 48 hours is characterized by the development of vacuoles in the cells. Until, or very nearly until, that stage is reached water absorption cannot be conditioned by osmotic forces, and the dominant factor must be imbibition. The values of Tables III and IV indicate that the water content rises rapidly during the first 24 hours, but that the rate of increase falls off between 24 and 48 hours. The course of the changing water content during this first phase suggests the operation of imbibition forces, with the water content approaching a limit at 48 hours. When vacuolation begins, further quantities of water are absorbed by osmosis, and the rise in water content which occurs after 48 hours is undoubtedly a reflection of the operation of this factor. Evidently the rate at which the water content increases after saturation by imbibition will depend on the rate at which dormant cells become active and new cells are formed. These are characteristic features of cotyledon development, and therefore water content, in view of the fact that dry weight does not increase, is probably as satisfactory

an index of growth as any other; for this purpose fresh weight could also be used. Water content, however, has the advantage over fresh weight that through it the variations due to the different initial dry weights are largely eliminated.

The treatments that establish the same relative differences for water content and area, occasion an entirely different order of dry weight changes. The curves of Fig. 2 show a fall in dry weight during the first 24 hours, which is particularly sharp where the pellicle has been removed. The difference may be due in part, but cannot be due entirely, to different respiration rates. From some unpublished data on the respiration rates of the cotyledon both with and without its pellicle it is possible to calculate the reduction in dry weight caused by this factor. The average respiration rate of a cotyledon without a pellicle during the first 24 hours is approximately 0·05 mgm. of CO_2 per hour. This would involve the utilization of about 0·8 mgm. of hexose sugar, but the decrease in weight approaches 7 mgm. Marel (1919) has shown that the pellicle has semipermeable properties. Therefore if solutes could diffuse out of the living tissues on the outer surface of the cotyledons their release into the surrounding water would depend upon the removal of the membrane. This suggests that the relatively large decrease in weight on the removal of the pellicle is due to the leaching out of solutes. This decrease in weight occurs not only when the surface from which the pellicle is removed is placed in contact with the substratum but also when it is uppermost. In the first case it is in contact with free water but not in the second, so it might be argued that if leaching is in fact the operative factor then it should occur only in the former case. The atmosphere above the beads in the Petri dishes is, however, always saturated, and a film of water into which diffusion could occur always forms over the upper cotyledon surface. With regard to the distinctions between the two surfaces it is significant that leaching occurs only from the outer. Where cotyledons retaining their pellicles are cultured and where therefore only the inner surface is free, there is no considerable reduction in dry weight whichever surface is placed in contact with the water.

The respiration rate continues to rise after 24 hours, but after the initial fall there is no further apparent decrease in weight, nor on the other hand is there any considerable increase. This suggests that there must be some factor operating which compensates for the decrease in weight to be expected from respiration. One such process is the conversion of insoluble fats and proteins into oxidizable carbohydrates. This process is known to occur and it might therefore contribute to the stabilization of the dry weight if the carbohydrate accumulates. Another factor which might influence the dry weight is the induction of photosynthesis, the cotyledons beginning to turn green at about 48 hours. The curves of Fig. 2 suggest, however, that the formation of chlorophyll does not immediately promote the development of active assimilation. There are no indications of any increase in weight until about 96 hours, and if there is assimilation during this time it must be at no greater rate than

that at the compensation point. The work of Briggs (1922) on the induction of photosynthesis in seedlings is of some interest in this connexion. He found that the cotyledons of Cucurbita, unlike those of Ricinus, assimilated immediately after they had shed the seed-coats. In the normal seedling cotyledon, however, this stage is reached some time later than that at which the isolated cotyledons turn green, and the apparent discrepancy may be due to this difference.

The removal of the pellicle from the outer surface where this is uppermost leads to increase in the water content of the cotyledons. This, for the reasons presented above, is taken to indicate an acceleration in general development. The pellicle can only exert an effect either by transmitting or preventing access of some substance to the cotyledons. It is possible that the pellicle carries or produces some soluble substance which diffuses into the cotyledon and acts as an inhibitor. The possibility was tested by supplying naked cotyledons with water in which pellicles had been soaked, and comparing their growth with that of others cultured on distilled water, but there was no difference between the two series. If the pellicle depresses development by restricting the exchange with the environment it may do so through any one of the following factors: (1) water, (2) oxygen, and (3) carbon dioxide.

As will be shown later, the effect due to the pellicle must be operative during about the first 48 hours, and the possible restrictive action of the pellicle on water absorption should therefore be apparent during this period. The data of these experiments provide no indication that the removal of this membrane increases the absorption of water in the early phase; on the contrary, the evidence suggests that this operation actually decreases water absorption.

On the other hand, evidence presented elsewhere (Brown, 1940) suggests that one of the effects of the pellicle may be that of restricting the gaseous exchange. The permeability of the wet membrane to oxygen, when the gas is diffusing from pure oxygen into another gas, is of the order of 0.7 c.c. per sq. cm. per hour. A partial pressure of this gas of a fifth of an atmosphere should maintain a diffusion rate across a membrane of average area of about 0.06 c.c. per hour. At 48 hours the naked cotyledon may consume as much as 0.25 c.c. of oxygen per hour, and assuming that half this quantity is normally absorbed by the outer surface, the total oxygen deficit when diffusion is maintained across the pellicle will be of the order of 0.065 c.c.

The resistance of the pellicle to the diffusion of O_2 is not less than four times as great as to that of CO_2. The same rate of diffusion of the gases will thus occur when the difference in concentration of carbon dioxide on the two sides of the membrane is a quarter of that of oxygen; such a difference in concentration will be established when the concentration of carbon dioxide within the membrane reaches 5 per cent.

Thus the presence of the pellicle undoubtedly reduces the oxygen supply and restricts the diffusion outwards of carbon dioxide; both of which are

conditions likely to depress the development of the surface concerned, namely the outer.

In the experiments shown in Fig. 1 and Table III the effect of the removal of the pellicle differs according as to which surface is uppermost. When the outer surface is uppermost the effect is considerable, but when on the other hand the inner surface is uppermost then the same treatment has little if any effect. In this connexion two of the conclusions drawn from the experiments in Table IV and of Figs. 3 and 4 are of some significance, (1) that the development of the inner surface is inhibited by high light, and (2) that of the outer surface is relatively depressed by light only when it is covered by the pellicle. Now when the outer surface is uppermost with the pellicle attached a double restriction is imposed; it is inhibited by light and also by an inadequate oxygen supply. When the pellicle is removed the double restriction is removed. In both cases the inner surface—which is relatively more light-sensitive—being directed downward, is in a relatively low light, and is not restricted in its development. Therefore when the pellicle is removed the growth of the whole cotyledon is promoted through the comparatively unrestricted development of both surfaces.

Now, when the inner surface is uppermost, this surface being adversely affected by the high light intensity must limit the growth of the cotyledon as a whole. Whatever the potential development of the outer surface, its effect on the cotyledon must remain limited by the inhibition imposed on the inner. Hence the comparatively negligible effect of the removal of the pellicle when the cotyledon is in this position.

The effects of the pellicle have been established by a comparison of the water content of the final samples. The pellicle, however, can only exert its effects during a comparatively limited period of the life history of the cotyledon. The pellicle does not grow, so that after its limited capacity for elastic extension has been reached the attachment between it and the cotyledon must be broken when the latter starts rapidly increasing in area. During the first phase of development, when the cotyledon increases mainly in thickness, no shearing stress develops between the pellicle and the outer surface. This must happen, however, when rapid area growth begins at about 48 hours, and the attachment will then be broken. Actual observation has shown that it is not until about this stage of development that the pellicle can be lifted easily from the outer surface.

Thus the effects that appear after the attachment between the cotyledon and the pellicle has been broken must be after-effects which were established in the earlier phase.

All the experiments reported in this paper were carried out in the light. Unfortunately only a few general observations were made on cotyledons grown in the dark. These, however, showed that although reducing the light increases growth, yet some light is necessary for development from the dormant to the active phase. In the dark the cotyledons apparently swell by

imbibition, but the transition to the active non-dormant phase occurs only very slowly.

SUMMARY

1. A method is described for the cultivation of the cotyledons of *Cucurbita Pepo* in isolation which ensures uniformity in the cultures and can therefore be used to obtain quantitative data.

2. The effect of the level of water availability, the effect of supplying water to the outer and to the inner surfaces, light intensity, and the presence and absence of the inner seed-coat membrane on the outer surface of the cotyledon have been investigated.

3. The rate of cotyledon development is always highest with the highest levels of water availability, and the rate falls as the level of water availability is decreased.

4. Evidence is presented which shows that the reaction of the cotyledon differs according to the surface exposed, and this, together with certain anatomical observations, suggests that the immediate effect of certain treatments is localized in the surface layers and the effect on the whole cotyledon is secondary.

5. Light depresses the development of the inner surface, but only affects the outer when it is covered by the inner seed-coat membrane.

6. The removal of the inner seed-coat membrane enhances development in light when the outer surface is turned upwards.

7. Some of the effects due to the removal of the inner seed-coat membrane are only evident after the connexion with the cotyledon has been broken. These are therefore after-effects which were established in an earlier phase.

8. The removal of the seed-coat occasions a sharp fall in dry weight which is attributed to a leaching out of soluble substances.

9. Two phases in the development of the cotyledon are distinguished. An early phase, extending over about forty-eight hours, in which water absorption is by imbibition; and a later phase in which absorption is by osmosis.

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Neuropteris tenuifolia with Carpons attached

BY

W. HEMINGWAY

With Plate IV

D URING the last decade evidence has been accumulating that some of the seed-like bodies found in Carboniferous rocks are actually micro-spore-bearing synangia. Some years ago I discovered spores in certain supposed seeds of the Rhabdocarpus type, and these, which were brought to the notice of the Botanical Congress at Cambridge in 1930 (Halle, 1931), were subsequently described in detail by Halle (1933) and referred to a new genus Aulacotheca. In dealing also with similar seed-like but spore-bearing bodies (e.g. Goldenbergia) Halle pointed out that these cases raise the question of whether other impressions or carbonized specimens that are generally believed to be seeds may be spore-bearing organs, though in some of the classic instances of supposed seeds actually attached to pteridospermic fronds he was unable to find any conclusive evidence.

Since it is not always possible to determine their exact nature, there is clearly a need for a new term applicable to all these seed-like bodies borne by the Upper Palaeozoic Pteridosperms, and I propose for them the name *carpon*. Nor is it simply a question of whether these carpons are seeds or synangia. Various morphological possibilities must be borne in mind, and a carpon might be (1) a simple seed; (2) a cupulate seed, possibly with the cupule opening only at maturity; (3) a seed with a concrescent cupule; (4) a cupule containing more than one seed; (5) a closed hollow microspore-bearing synangium, perhaps opening apically at maturity; (6) an open cup-like synangium (though this type would be less seed-like); or (7) a microspore-bearing synangial cupule enclosing a seed or seeds.

The present paper deals with one hitherto unrecorded case of carpons attached to foliage. Many years ago Mr. W. R. Barker, of Barnsley, Yorkshire, sent me three specimens of a Neuropteris with carpons attached. The foliage on these specimens was in a very immature condition and could not be satis-factorily identified at the time. The specimens had been found in the Monck-ton Rock at Monckton Main Colliery, Royston, Barnsley. After many visits to this colliery in the hope that additional and better specimens would be found, I had the good fortune to visit the place at a time when a considerable quantity of shale had been raised to the surface. This shale was full of more or less broken up portions of *Neuropteris tenuifolia* intermingled with many

carpons identical with those seen attached to Mr. Barker's specimens. Fortunately there was no admixture of other species included in the mass. After a long search in this material I succeeded in finding a large slab of shale on which were eight carpons, two of which were attached to a branching stem bearing identifiable leaves (Pl. IV, Figs. 2, 2*a*). These satisfactorily established the fact that the carpons belonged to *Neuropteris tenuifolia* Schlotheim. This species occurs sparingly throughout the British Coal Measures from the Kilburn Coal, Lower Yorkian, up to the Red Beds of Conisbro', Upper Yorkian. Several additional specimens with carpons attached have now been found, and the identity of the plant and its carpons fully confirmed. The foliage in this shale varied from young pinnae with very small pinnules to portions of fronds with fully developed pinnules, but in all cases where the carpons are attached the foliage is in a very immature condition.

The appearance of the carpons is sufficiently indicated in the figure of a detached carpon (Pl. IV, Figs. 3, 3*a*); it is about 2 cm. in length and 1 cm. in breadth. Pl. IV, Fig. 1, is a photograph, natural size, of one of Mr. Barker's specimens. The carpon is fully developed while the foliage is still in a very young stage, merely a confused mass of crumpled leaves. The stem to which the carpon is attached can be traced through the leafy mass to the outstanding leaf at the base of the specimen and appears to have borne about seven leaves. In Fig. 6 of this plate there is shown a composite photograph designed to illustrate more clearly the manner of attachment of leaves and ··carpon in Fig. 1. It will be noted that the carpon occupies the position of the terminal pinnule, as is the case in all examples yet observed.

Two carpons attached to a branching stem with fragments of leaves below the carpons are to be seen in Pl. IV, Fig. 2, and Fig. 2*a* shows the same enlarged two and a half diameters to exhibit more clearly the two overlapping carpons and the fragments of leaves at the base. The composite photograph (Pl. IV, Fig. 7) gives a suggested interpretation of this specimen with the two adjacent carpons terminating two very short lateral pinnae.

The carpons of *Neuropteris tenuifolia* appear to be of the nature of a tripartite cupule which split open into three segments or valves, exposing a nucule. A specimen shown on Pl. IV, Fig. 4, appears to be a separated segment or valve and shows the small central nucule very clearly. Pl. IV, Fig. 4*a*, is an enlargement of this specimen to show its characters more clearly. The separated valves are narrower in proportion to their length than when the entire carpon is preserved. The nucules vary in size from 4 mm. by 2 mm., as in the figured specimen, to 8 mm. by 4 mm.; this variation is possibly due to the degree of development. We have as yet no evidence as to the nature of the little nucules. Macerated specimens have so far only given negative results. At present I favour the view of their being seeds.

Some fine examples of these carpons found in the little ironstone nodules in the roof of the Barnsley Coal at Worsbro', near Barnsley, are uncompressed, i.e. preserved in the round, and show the presence of three wings. The

detached carpon shown enlarged in Pl. IV, Fig. 3*b*, illustrates the transverse barring of the wings. The carpons are identical with certain 'seeds' which have been referred to the genus Polypterocarpus Grand' Eury, some of which are about the same size, e.g. *P. ornatus* (Kidston). The separate valve or segment shown in Pl. IV, Figs. 4, 4*a*, may be compared with the figure of *Polypterocarpus anglicus* (Arber) given by Kidston (1914, Pl. XIV, Fig. 1) under the name of *Tripterospermum ellipticum*, but Kidston's 'seed' was 6 cm. long and therefore very much larger than the carpons now under consideration. (For the nomenclature of this species, see Seward, 1917, p. 357.) When preserved in an uncompressed condition, these carpons have a considerable resemblance to *Trigonocarpon parkinsoni*, but may be distinguished by the large and circular basal scar of the latter; in the carpons of *Neuropteris tenuifolia* there is no scar but only a small triangular cavity extending into the interior of the carpon, probably resulting from the decay of the vascular strand.

During the past twenty years I have examined many fine examples of Pteridosperm carpons attached to their foliage branches, and in nearly all cases the foliage has been in a very young and undeveloped stage, often in a circinate condition. I have only once met with a carpon (*Neurocarpon heterophyllum*) attached to a large well-developed frond. From this fact it would appear that the fructifications were produced on young fronds and that as the fronds opened out the fruits fell off. This may account for the rarity of specimens bearing fruits in our museums and cabinets, for only under exceptional circumstances could fruit-bearing fronds be fossilized.

I have to thank the Royal Society for grants to help defray the costs of this research, and Mr. W. N. Edwards of the Geology Department of the British Museum (Natural History) for assistance in the preparation of the paper.

SUMMARY

1. The term *carpon* is proposed for the terminal seed-like fructifications of Pteridosperms.

2. Carpons attached to *Neuropteris tenuifolia* are described; in this case they may possibly have been tripartite cupules enclosing a seed.

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EXPLANATION OF PLATE IV

Illustrating Mr. Hemingway's paper on '*Neuropteris tenuifolia* with Carpons attached'.

All the figures are from specimens in the writer's collection and are from the Barnsley Coal. Yorkian Series, Monckton Main Colliery, near Barnsley, Yorkshire.

Figs. 6 and 7 are composite photographs intended as restorations.

Fig. 1. A fully developed carpon attached to a leafy stem in the midst of a bunch of imperfect foliage. Natural size. Specimen WH/2141. (Cf. also Fig. 6.)

Figs. 2 and 2a. Two overlapping attached carpons, with fragments of leaves below. Fig. 2 is natural size. Specimen WH/2330. Fig. 2a is magnified 2·5 diameters. (Cf. also Fig. 7.)

Figs. 3, 3a, and 3b. Complete carpon flattened in shale. Natural size. Specimen WH/2201. Fig. 3a, magnified nearly 2 diameters; Fig. 3b, magnified 2·5 diameters and lit from a different angle, so as to show the transverse barring on the wings.

Figs. 4 and 4a. Apparently a carpon split open, showing the small nucule in the centre. Fig. 4 is natural size. Specimen WH/2216. Fig. 4a is magnified 3 diameters.

Fig. 5. Fragment of a pinna to show the terminal pinnule which corresponds to the position of the carpons. Natural size. Specimen WH/2330.

Fig. 6. Composite photograph designed to illustrate more clearly the attachment of the carpon to the leafy branch in Fig. 1.

Fig. 7. Composite photograph designed to illustrate the manner of attachment of the carpons and leaves in specimen WH/2330 (Fig. 2).

On the Coal-Measure Plant, Aulacotheca

BY

W. HEMINGWAY

With Plate V

THE genus Aulacotheca was devised by Halle (1933) for the reception of some elongated, fluted, seed-like bodies containing spores of the Whittleseya type.

In investigating the fossil plants of the Yorkshire and Lancashire Coalfield I have found a large number of similar bodies, which I have recently called *carpons* (Hemingway, 1941). These varied considerably in size and other characters, but had all been included by Kidston in his species *Rhabdocarpus elongatus*. From the differences observed, and the different plants with which they were associated, it became probable that we were dealing with a group of closely similar forms rather than with a single species.

Halle distinguished three species: *A. elongata* (Kidston) from the Yorkian of Scotland; *A. hemingwayi* Halle from the Yorkian of Yorkshire, Staffordshire, and Nottinghamshire; and *A. idelbergeri* Halle from the Westphalian of Gelsenkirchen.

This paper is an attempt to carry further the separation of some of the constituents of the group.

The forms comprised in Aulacotheca are almost invariably found associated with the fronds of Alethopteris, usually some form of the *A. lonchitica* group. No reliable case of attachment to foliage has yet been found. Probably these structures were produced at a very early stage in the plant's development and on a differentiated shoot as in Potoniea, though Potoniea belongs to the small-spore division of the Pteridosperms. Possibly the carpons became detached before the foliage opened out. If this was the case it explains why we never find them attached to the fronds or foliage branches.

Many instances have been observed where the carpons are scattered broadcast along with the foliage; any apparent attachment may be only accidental. When a large number of fragments of foliage and carpons are scattered near each other it is quite likely that some may fall in such closeness as to simulate organic connexion, as in the case of *A. dixiana* described below.

The pollen-grains (or spores) in Aulacotheca are very large oval bodies with a longitudinal germinal slit. They vary in size in the several species from 100 to 300 μ in length—very much larger than those of the microspore division of Pteridosperms, which rarely exceed 50 μ in diameter. This difference

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in size of the spores seems to indicate the existence of two widely different families in the Pteridosperms.

AULACOTHECA ELONGATA (Kidston).

Syn. *Rhabdocarpus elongatus* Kidston. This species is excellently described by Halle (1933). It comes from the lower Yorkian of Airdrie, Lanarkshire.

AULACOTHECA HEMINGWAYI Halle (Pl. V, Figs. 1–9).

This species is the best known of the series, and is also well described by Halle (1933). It is very common in the middle Yorkian Series of the Yorkshire and Derbyshire Coal-fields, and in the Staffordshire and Northumberland Coal Measures.

It is a 9-locular capsule, the loculi being filled with large oval pollen-grains 190–210 μ long and 105–17 μ broad with a longitudinal germinal furrow. In the split-open capsules the pollen-grains lie in broad bands corresponding to the loculi. In these bands of spores the grains seem to be more or less in lines. This supports a view I have held for some time, viz. that the loculi were not merely enlarged sporangia but were synangia and that each loculus originally contained a bundle of long slender sporangia. In Boulaya the sporangia are in three bundles separated by bands of barren tissue, the sporangia probably embedded in some kind of tissue as in Dolerotheca. This point requires much further research before one could say definitely that the loculi of Aulacotheca were a *ring of synangia* and not merely a ring of glorified sporangia.

One interesting specimen (Pl. V, Fig. 9) shows, alongside a carpon, a tortuous trail of pollen-grains. Pl. V, Figs. 3, 4, and 5, show carpons split open revealing bands of pollen-grains *in situ*, together with the characteristic dark band of tissue along the central line. Pl. V, Fig. 7, is a diagrammatic transverse section of a sporocarp. The dark band in the centre of Pl. V, Figs. 3, 4, and 5, may, as suggested by Halle, be the flattened inner angles of the loculi. The pollen-grains in these carpons are very numerous; in a specimen recently broken open the contents fell out as a loose powder which was found to consist of pollen-grains and decayed tissue, the grains being estimated to be over 5,000.

A good example of a carpon as it occurs in coal shale is shown in Pl. V, Fig. 1. Four strongly developed ribs and furrows, the outer evidence of the loculi in the interior, are seen on the flattened surface; on the surface of the ribs there are four smaller ribs which may possibly indicate that the loculi contained long slender sporangia—15–20 in each loculus, as suggested above.

The pointed end of the carpon is very slender and wavy; it is believed that the organs were attached by this pointed end, but it is difficult to understand how such a comparatively large object could be supported by such a fine

point. Possibly they were contained in some kind of a cupule and supported each other; no cupule, however, has been observed, so we can only conjecture as to their mode of attachment.

The specimen shown in Pl. V, Fig. 8, and one figured by Halle (1933, Pl. VIII, Fig. 2), show a number of sporocarps with their pointed ends all in one direction; two or three other specimens with the sporocarps similarly orientated have been found. Pl. V, Figs. 2, 2a, show what at first sight looks like a stalk attached to the broad end, but I think the apparent stalk is just an accidental bit of stem lying near the large end and is in no way connected with the carpon.

Aulacotheca hemingwayi is invariably associated with the frond *Alethopteris decurrens* (Artis). It is, I believe, the male fructification of this plant, and I have collected many specimens which show the carpons and foliage on the same slab of shale.

AULACOTHECA DIXIANA, n.sp. (Pl. V, Figs. 10, 10a).

Dix (1932) described a specimen of this carpon apparently attached to *Alethopteris rectinervis* Kidston. The position of the carpon seems an unnatural one in relation to the pinna, and as this is the only specimen of its kind seen, we are justified in regarding it as an accidental case of juxtaposition.

Halle felt dubious about the connexion, and Mr. W. N. Edwards informs me that a recent re-examination of the specimen, now in the British Museum (Natural History), by improved methods of lighting showed conclusively that there was no attachment. This is indeed suggested by the photograph published by Dix (1933, Pl. XXI, Figs. 66, 66A) subsequently to her original note.

Pl. V, Fig. 10, shows the manner in which this carpon occurs in association with the scattered pinnules of *Alethopteris* (*Neuropteris*) *rectinervis*. Pl. V, Fig. 10a, is the same enlarged two and a half diameters to show the surface striation. *Aulacotheca dixiana* appears to be 9-loculed, but the ridges are not so clear and pronounced as in *A. hemingwayi*. The spores, which can be separated by maceration, are said to be slightly smaller than those of *A. hemingwayi*.

This species comes from the Millstone Grit Series of Northumberland, where it occurs in close association with a plant which I identify as *Alethopteris rectinervis* (Kidston). Dix names it *Neuropteris schlehani* Stur. Similar plants occur in profusion in the lower Yorkian rocks of Yorkshire and Derbyshire, also in Wales and in the Culmian of Devonshire, but as far as I know no specimens of *Aulacotheca dixiana* have been found at any of these localities. It is probable that more than one species may be included under the names *Neuropteris* (*A.*) *schlehani* and *Alethopteris* (*N.*) *rectinervis*. These plants belong to the *Alethopteris lonchitica* group and require a careful revision.

I have pleasure in naming this species after Dr. Emily Dix who first described it.

AULACOTHECA HALLEI, n.sp. (Pl. V, Figs. 11, 11*a*, 11*b*). Type of species.

This species differs from those previously described in its smaller size, in the dimensions of the spores, and in having apparently only six loculi.

Pl. V, Figs. 11 and 11*a*, show part and counterpart of the carpon natural size, specimen WH/2361 (Hemingway Collection); the nodule is split open showing the interior filled with spores and calcitic matter. It is from the 10 ft. ironstone beds, Middle Yorkian, Coseley, Dudley, Staffordshire. The carpon is 16 mm. long and 3·5 mm. broad. The spores are oval, 113×73 μ in size, and have the longitudinal germinal suture characteristic of the Whittleseya group. The interior is preserved in the same manner as the specimens shown in Pl. V, Figs. 3, 4, and 5. The spores appear to lie in 6 loculi, and no central columella-like structure is visible.

I name this species after Professor T. G. Halle.

I have pleasure in thanking Mr. W. N. Edwards of the Geology Department, British Museum (Natural History), for assistance in the preparation of the paper.

SUMMARY

The species of Aulacotheca, a spore-bearing carpon from the Coal Measures, are reviewed. A new species, *A. Hallei*, is described and an unnamed carpon originally described by Dr. E. Dix is referred to this genus and named *A. Dixiana*.

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EXPLANATION OF PLATE V

Illustrating Mr. Hemingway's paper 'On the Coal-Measure Plant, Aulacotheca'.

Figs. 1–9, *Aulacotheca Hemingwayi* Halle.

Fig. 1. A carpon as it occurs flattened in shale at Monckton Main Colliery, Royston, Yorkshire. Hor., Barnsley Coal, Mid-Yorkian. Natural size. (Hemingway Collection, WH/2301.)

Fig. 1*a*. The same, enlarged 2½ diameters to show the ribs and riblets more clearly. The projection at the large end is only a stain on the stone and is not an attachment to the carpon.

Fig. 2. Carpon, with what looks like a stalk attached to the large end; this is probably accidental. From a Dudley nodule, Mid-Yorkian, Staffordshire. (Hemingway Collection, WH/2342.)

Figs. 3, 3*a*, 4, 5, 5*a*. Specimens in the Hemingway Collection from Dudley nodules, showing pollen-grains *in situ* and the central dark band of tissue. Figs. 3, 4, 5, and 5*a* natural size; 3*a* enlarged 3 diameters. These specimens are split in two showing the spores in calcitic matrix. Mid-Yorkian, Dudley, Staffs.

Fig. 6. Very slender carpon, which might be a distinct species. 10-ft. Ironstone Measures, Dudley.

Fig. 7. Diagrammatic transverse section showing the 9 primary ridges and grooves, also the secondary ridges and the thick inner angles of the loculi.

Fig. 8. Another example, natural size, from a Dudley nodule, showing the jointed ends in one direction.

Fig. 9. From a Dudley nodule, showing a carpon and a curious tortuous trail of pollen-grains alongside. Natural size. 10-ft. Ironstone Measures, Mid-Yorkian, Dudley, Staffs. (Hemingway Collection, WH/2365.)

Fig. 10. *Aulacotheca Dixiana.* Natural size. Associated with pinnules of *Alethopteris rectinervis* Kidston. Millstone Grit Series, Shilford Colliery, Northumberland. (Hemingway Collection, WH/2355.)

Fig. 10a. The same carpon. Enlarged nearly 3 diameters, to show shape and striation of surface. (Hemingway Collection, WH/2355.)

Figs. 11 and 11a. *Aulacotheca Hallei.* Natural size (part and counterpart) split open showing the interior filled with pollen-grains. 10-ft. Ironstone Measures, Coseley, Staffordshire Mid-Yorkian. (Hemingway Collection, WH/2361.)

Fig. 11b. The same. Enlarged 3 diameters to show the interior and spores more clearly.

HEMINGWAY — AULACOTHECA.

Polyploidy, Crossing-over, and Heterochromatin in Paris

BY

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With eight Figures in the Text

1. SCOPE OF VARIATION

THE genus Paris includes six species known in life (apart from thirty others known in books). Four of these are diploid, two of them having clonal auto-triploid forms. The two others are tetraploid and octoploid (Table I). All of them have the same five types of chromosomes recognizable at mitosis. All of them, so far as we know, have proximally localized chiasmata at meiosis. One of them, *P. polyphylla*, is remarkable for having large distal segments which appear as heterochromatin in mitosis, that is to say they are overstained in resting nuclei and, after freezing, understained in metaphase. This species has no nucleolar organizers. In these two respects it resembles the diploid species of Trillium and probably differs from other species of Paris.

Within this genus there are therefore differences in regard to polyploidy, nucleolar organization, and heterochromatin. And these differences occur in a group with a special distribution of crossing-over. A correlated study of the group is therefore likely to reveal a relationship, either inherent or adaptive, between four hitherto unrelated chromosome functions.

TABLE I   *Species of Paris*

Species.	Distribution.	Ploidy.	Chiasmata.	Author.
1. *P. polyphylla*	Himalaya	2x	P	Darlington and La Cour, 1938
2. *P. tetraphylla*	Japan	2x	—	Stow, 1935
3. *P. hexaphylla*	,,	2x	P	Haga, 1937*a*
	,,	3x	P	,,
4. *P. obovata*[1]	,,	2x	P	Haga, 1934
	,,	3x	—	
5. *P. quadrifolia*	Europe	4x	P	Darlington, 1937; Geitler, 1938
6. *P. japonica*[2]	Japan	8x	P	Haga, 1937

P = proximal localization of chiasmata at first metaphase in the pollen mother-cell.

[1] As *P. quadrifolia* var. *obovata* but cytologically indistinguishable from *P. hexaphylla*.   [2] As *Kinagusa japonica*.

The preparations of the pollen mother-cells were medium Flemming and La Cour's 2 BE stained with iodine—gentian-violet. Those of the pollen-grains and roots were acetic-alcohol—Feulgen smears. For both I am indebted to Mr. La Cour.

2. DIPLOID AND TETRAPLOID CHROMOSOMES

The chromosomes of the tetraploid *P. quadrifolia* fall into the usual five
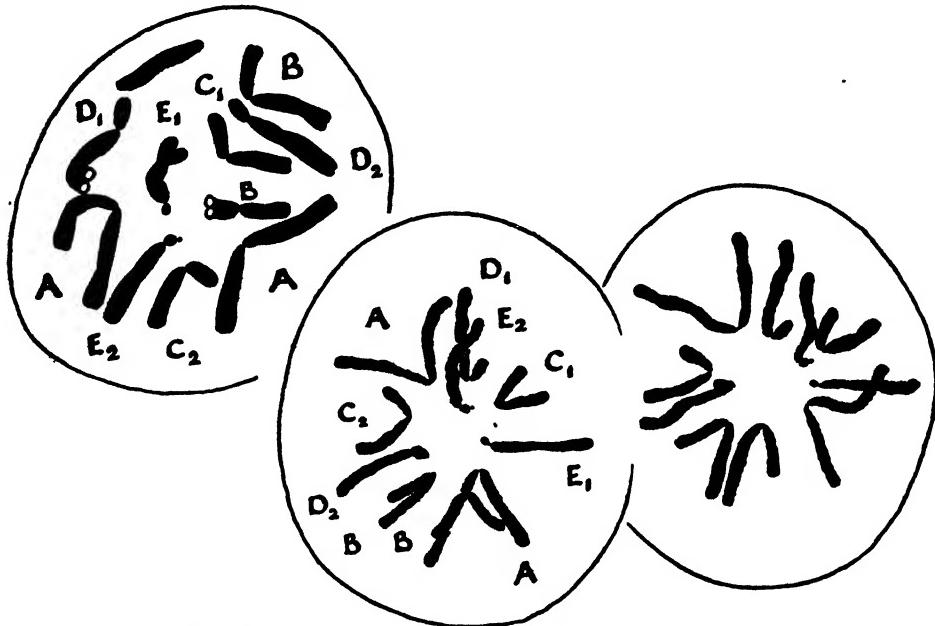
FIG. 1. Metaphase and anaphase of first pollen-grain mitosis in *Paris quadrifolia*; acetic-alcohol—Feulgen smear. × 1800.

types, the fourth and fifth (*D* and *E*) having subterminal centromeres. Structural differences and structural hybridity have been found in several species of Paris. Inversions in *P. quadrifolia* (Geitler, 1938), a deficiency and a fragment in *P. polyphylla* (Darlington and La Cour, 1938), and deficiencies in the smallest segment of the nucleolar chromosomes (where alone they could be detected) in *P. obovata* and *P. hexaphylla* (Stow, 1935; Haga, 1937a) alike warn us of the effects of structural variation in the group. The external uniformity of the five types of chromosomes shown by a comparison of the species may therefore well conceal a great deal of internal rearrangement.

Comparison in fact shows that in their shorter arms the four members of each type in *P. quadrifolia* differ recognizably in the way to be expected in an allopolyploid (Fig. 1). The most important difference is in the *E* chromosome. E_2, with the larger short arm, has a terminal nucleolar organizer which appears as a *seta* at metaphase. E_1 has no organizer or seta. This tetraploid species therefore resembles *Hyacinthus orientalis* in having a superficially tetraploid

TABLE II

Lengths of Chromosomes in Three Species of Paris from Mitosis in the Root-tips
(in microns)

Chromosomes.	A.	B.	C.	D.	E.	Total.
1. *P. polyphylla* 2x	19+17	17+12	15+13	22+1·5	20+2	138·5
(D. and La Cour,	*36*	*29*	*28*	*23·5*	*22*	—
1938)						
2. *P. obovata* 2x	17+16·5	16+10·5	11+8·5	17+5·5	19+1·5	122·5
(Haga, 1934)	*33·5*	*26·5*	*19·5*	*22·5*	*20·5*	—
3. *P. quadrifolia* 4x	9·5+9	7·5+5	5·5+3·5	9+2·5	9+0·5	61·0
(average of 2 sets)	*18·5*	*12·5*	*9·0*	*11·5*	*9·5*	—
x 2	*37·0*	*25*	*18*	*23*	*19*	122·0

1 and 3, acetic-alcohol—Feulgen smears; 2, Flemming gentian-violet sections.
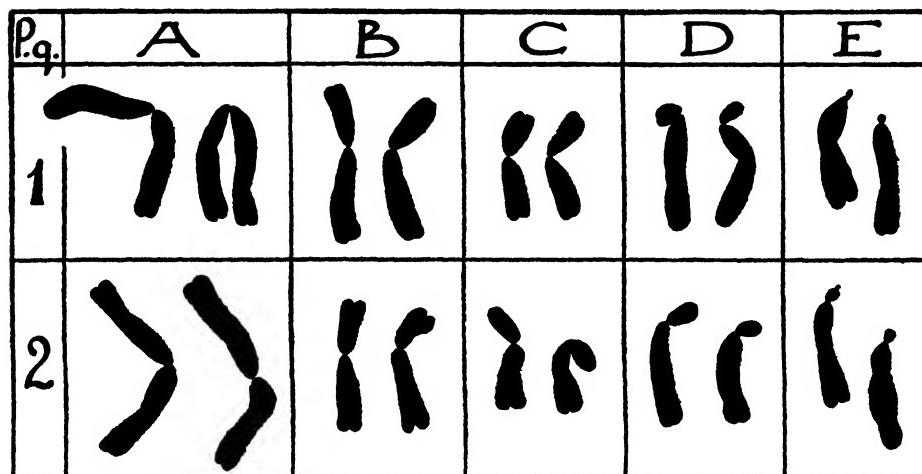
FIG. 2. Metaphase chromosomes from root-tip of *P. quadrifolia* showing the slight differences between the first and second sets; acetic-alcohol—Feulgen smear. × 1800.

complement but only one pair of nucleolar constrictions in its somatic chromosomes. No doubt a similar readjustment of nucleolar organization frequently occurs in the adaptation of new allopolyploid species (Darlington, 1926).

Apart from this difference, however, there are two other profound changes from the diploid species. The total lengths of the five types are half those in the nearest diploid (Table II). Since the cross-section of the chromatids is, if anything, also narrower in the metaphases we have used for comparison, the total volume of chromosome in the tetraploid is less than in the diploid. The genotypically controlled reduction in size more than compensates for the doubling of the chromosome number. Since the individual chromosomes of the diploids are near the limit of size found in any organism, we may suppose that the reduction of individual size was in this case a condition of the increase in total number.

That chromosome size and cell size are in fact related is illustrated very

well by comparing the pollen-grains in the tetraploid and octoploid species, for the pollen-grains in Paris are a particularly close fit (Table III). The chromosomes of *P. japonica* remain almost as large as those of the diploid species, and their pollen-grains are nearly four times the volume. They have

TABLE III

Species.	Ploidy.	Longest chromosome (root-tip).	Diam. of P.G.	Hetero-chromatin.	Nucleolar organizer.
		μ	μ	per cent.	
Trillium spp.	2x	30	30–40	10–15	0
Paris polyphylla	2x	36	—	12	0
P. spp. (Japan)	2x	34	40–50	?	2
P. quadrifolia	4x	18·5	33	0	2
P. japonica	8x	30	72	1	3

retained the original enlargement that went with the increase of the chromosome number. The pollen-grains of *P. quadrifolia*, on the other hand, are smaller than those of the related diploid species, and the whole bulk of their chromosomes is accordingly reduced. More extensive measurements would be worth while.
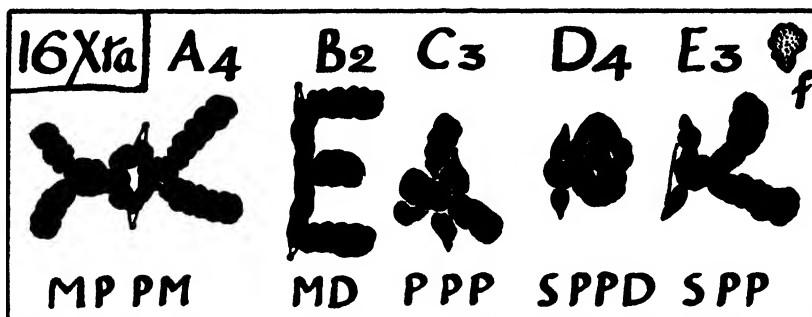
FIG. 3. First metaphase in p.m.c. of *P. polyphylla*, showing distribution of chiasmata. Flemming gentian-violet smear. × 1800.

The other difference is in the presence of heterochromatin. *P. polyphylla*, again like the *Trillium* diploids, has heterochromatic segments which appear overstrained in resting nuclei and show nucleic acid starvation at metaphase after freezing. In *P. quadrifolia* neither root-tips nor pollen-grains show the first property, and in root-tips La Cour has failed to find the second. This absence of allocyclic behaviour goes with the presence of nucleolar organizers. Within this group there may therefore be a correlation between the two although in one species of Fritillaria allocycly and organizers are found together (Darlington and La Cour, 1941).

3. MEIOSIS IN THE DIPLOID

A glance at the pollen mother-cells shows that in all five bivalents chiasmata in *Paris polyphylla* are proximally localized. The inequality to be expected

from the terminal deficiency of heterochromatin in one *C* chromosome, seen in the root-tips, was not apparent at meiosis in the anthers. From the frequency of such losses at mitosis in Trillium we may suppose that the roots alone had undergone this loss. We may even suppose on the analogy of
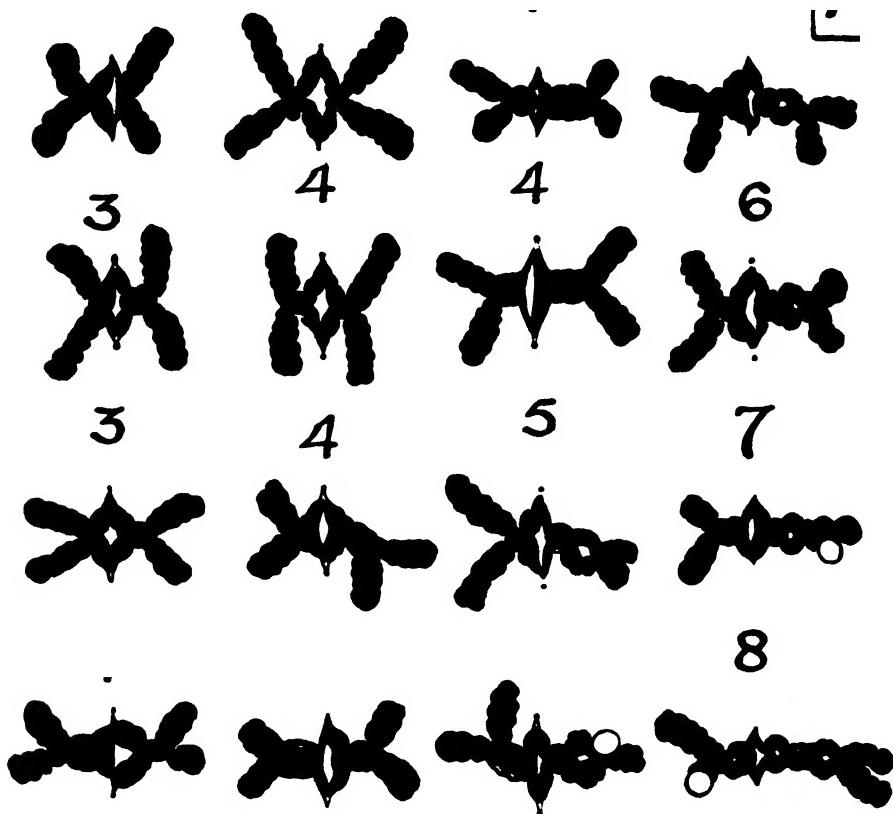
FIG. 4. Distributions of chiasmata in the A bivalent showing how the pairing must have extended from original contact points near the centromere. × 1800.

Sorghum (Janaki-Ammal, 1940) that the roots alone could afford to undergo such a loss.

The extra fragment as in Secale and Zea shows no relationship with the major chromosomes. When single it forms no chiasmata. Reduplicated by accident in one cell it formed a bivalent with a chiasma on either side of the centromere. Unlike the Zea fragments, it is not heterochromatic.

The chiasma frequency of the major chromosomes varies from o to 8 (Table III) and when a particular type, like *A* in Fig. 4, is taken and arranged in a series with increasing chiasma-frequency, it shows the characteristic

consequences of procentric pairing. Single chiasmata are always close to the centromere. Additional ones lie farther and farther away. Evidently pairing begins, as in Fritillaria (Frankel, 1940), near the centromere and proceeds along the chromosome towards both ends. The same is true of the *S* types,
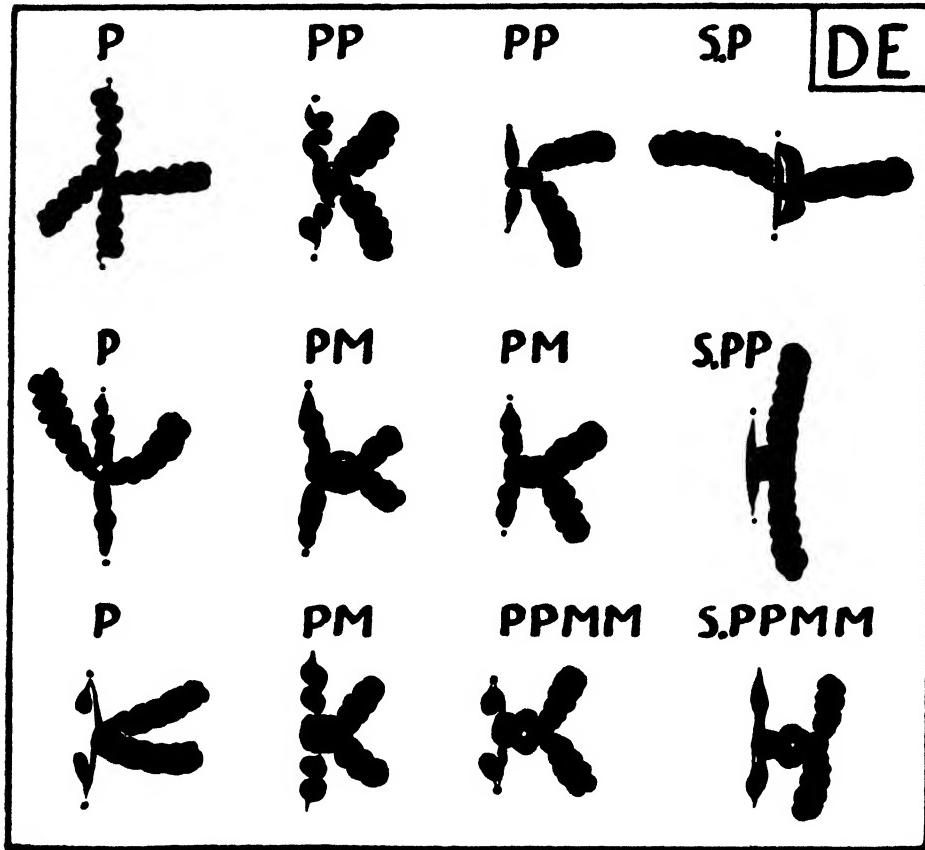
FIG. 5. Distribution of chiasmata in D–E bivalents. × 1800.

D and *E*, which are taken together since they are not regularly distinguishable (Fig. 5).

Various special consequences of procentric pairing in long chromosomes follow. The S-type chromosomes (*D* and *E*) have a higher chiasma-frequency than an M-type chromosome (*C*) which is longer (Table IV). This anomaly, however, is less striking than in Fritillaria. The shorter M-type chromosomes (*C*) have more frequent distal chiasmata. Pairing, we may suppose, is nearly complete for them at a time when it is still beginning for the longer chromosomes. There are, too, several minor differences from the Fritillaria type of localization:

(·1) The proximal chiasma is usually farther from the centromere, and the

centric loop is consequently larger. The differential distance, to use Mather's term (1939), is longer.

(2) Single-chiasma D and E bivalents never have their chiasma in the short arm. Pairing therefore never begins in the short arm.

(3) Hence the number of chiasmata in the long arm should be independent of their occurrence in the short arm. In fact D and E bivalents with a short-arm chiasma have the same number of chiasmata as, or even more than, those without a chiasma in the short arm (2·4 against 2·25).

TABLE IV
P. polyphylla, 20 cells

Chromosome.	Chiasmata.	0.	1.	2.	3.	4.	5.	6.	7.	Total.
A	Arms	—	9	24	5	1	1	—	—	40
	Wholes	—	—	2	4	8	4	1	1	20
B	Arms	1	15	22	2	—	—	—	—	40
	Wholes	—	—	3	10	6	1	—	—	20
C	Arms	3	18	18	1	—	—	—	—	40
	Wholes	1	—	5	9	5	—	—	—	20
	No s.a. chiasma	—	2	6	3	1	—	—	—	12
DE	One s.a. chiasma	—	3	13	10	2	—	—	—	28
	Total	—	2	9	16	11	2	—	—	40
ABC	Arms	4	42	64	8	1	1	—	—	120
	Wholes	1	—	10	23	19	5	1	1	60

The reason for these differences appears from a further splitting up of the data. The positions of all the chiasmata in all the bivalents of twenty cells can be recorded as between the short-arms (for D and E) and the proximal, median, and distal thirds of the long arms (for all chromosomes). The distribution of chiasmata in bivalents with different numbers of total chiasmata can then be separately classified for each type of chromosome (Table VI and Fig. 6).

TABLE V
Numbers of Chiasmata in Segments of Whole Bivalents

Chr.	S.	P.	M.	D.	Bivs.	Xta.	X/B.	Length. μ
A	—	43	33	5	20	81	4·05	36
B	—	23	34	8	20	65	3·25	29
C	—	22	23	12	20	57	2·85	28
DE	28	60	32	2	40	122	3·05	23
Total	—	—	—	—	100	325	3·25	—

The result is to show the difference in pairing and crossing-over mechanism between the most distinct types of chromosome. Localization in the proximal segment is clear in all types but much more marked in D and E than in the
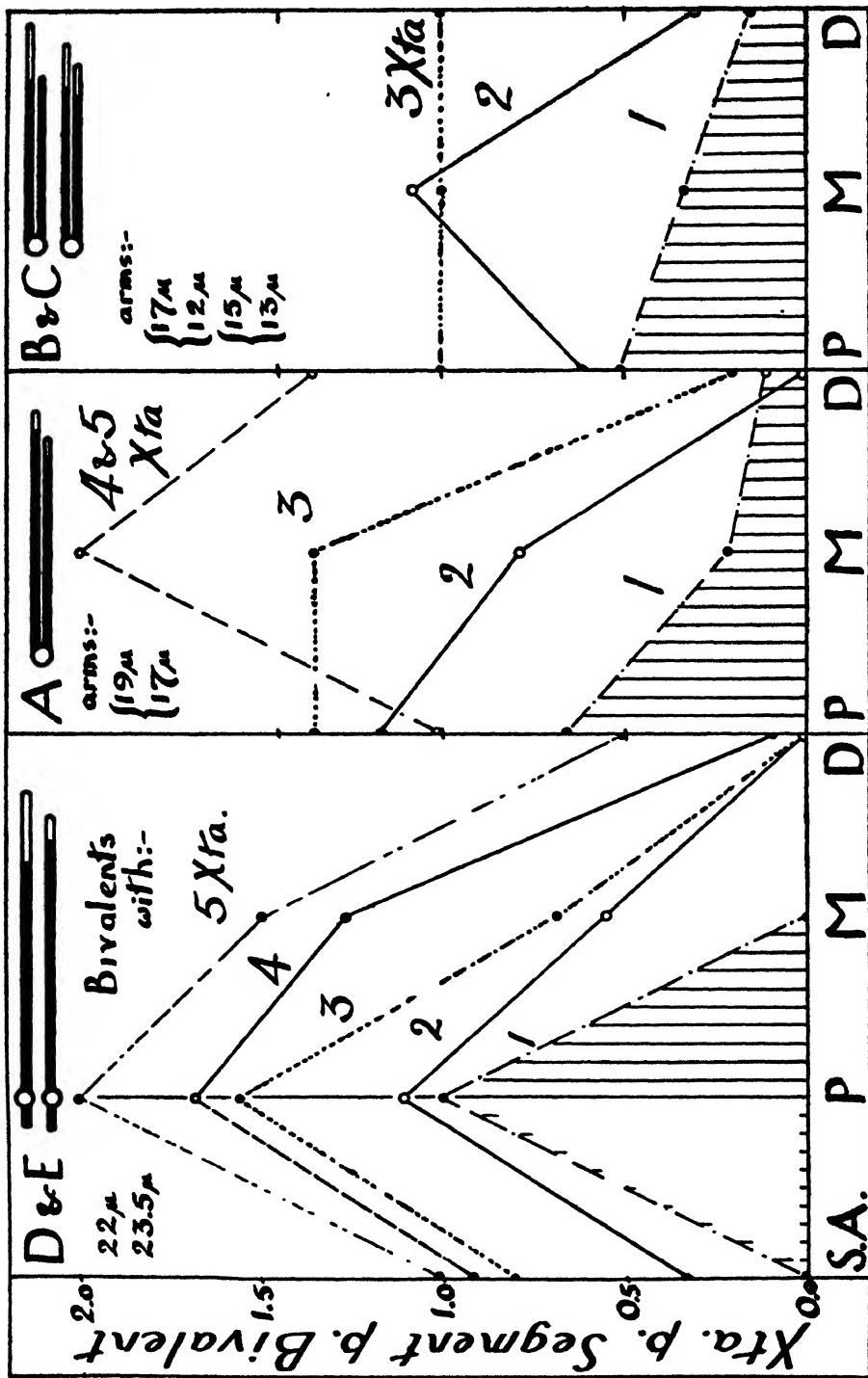
FIG. 6. Graph giving data of Table VII, per bivalent: *P. polyphylla.*

nearly equal-armed chromosomes. It is also more marked in the longer *A* than in the shorter *B* and *C*, for the reason we have seen. The *priority* of the long arms over the short arms of *D* and *E* is maintained in each class. This reinforces the conclusion that pairing regularly begins in the long arm and
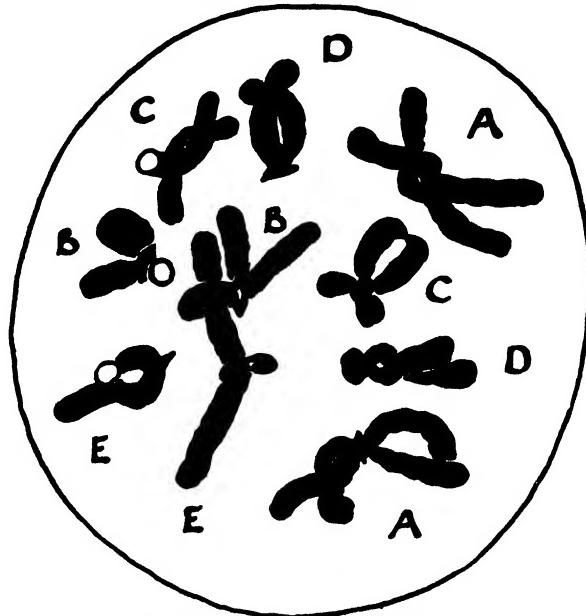
FIG. 7. First metaphase of meiosis in *P. quadrifolia*. ×2200.

TABLE VI

P. polyphylla. (Data in Fig. 6)

DE bivalents.	S.	P.	M.	D.	T. bivs.	T. Xta.
1 X . .	—	2	—	—	*2*	2
2 Xta . .	3	10	5	—	*9*	18
3 Xta . .	13	25	10	—	*16*	48
4 Xta . .	10	19	14	1	*11*	44
5 Xta . .	2	4	3	1	*2*	10
A Arms					T. Arms	
1 X . .	—	6	2	1	*9*	9
2 Xta . .	—	28	20	—	*24*	48
3 Xta . .	—	.7	7	1	*5*	15
4 & 5 Xta .	—	2	4	3	*2*	9
B C Arms					T. Arms	
1 X . .	—	17	11	5	*33*	33
2 Xta . .	—	25	43	12	*40*	80
3 Xta . .	—	3	3	3	*3*	9

not indifferently on either side of the centromere. In *A* the drift away from the centromere as a first contact point is more pronounced, and we even have one single-chiasma bivalent with the chiasma in the distal segment. In fact

the frequency of chiasmata in this segment is slightly higher than in the two-chiasma bivalents.

Now for general purposes we can take the successive curves as representing a succession of stages in pairing. The reduction of chiasmata in one segment of *A* is inconsistent with this rule and the inconsistency is repeated in the

TABLE VII

Variances Between and Within Arms (from data in Table I)

	A.	B.	C.	Joint.
Between Arms . .	0·76	0·31	0·49	0·52
Within Arms . .	0·63	0·48	0·43	0·51

tetraploid species (Fig. 8). Evidently the succession is not a strict one. The one-chiasma bivalents of *A* are those which have paired later or more slowly than the higher classes. The position of the chiasma will therefore reflect this difference in sampling. When the single chiasma is distal, pairing has begun distally, and so it may well be that when it does so pairing is often so late or so slow that a second chiasma fails to be formed. The succession is therefore true only for bivalents with similar contact points in the beginning of pairing.

There are two other inconsistencies: too few proximal chiasmata in the 4–5 chiasma class of *A*, and too many median chiasmata in the 2-chiasma class of *B* and *C*. These differences are probably due to inexact apportioning, as between segments, of chiasmata that are close together.

Mather has determined the variances within and between nuclei and finds no evidence of competition. An absence of competition is perhaps in this case due to limitation of pairing. His analysis also shows (Table VII) that chiasma-formation is completely independent in the arms of each *A*, *B*, or *C* chromosome, just as we saw it was for *D* and *E*. There is no significant difference between the Between and Within Arms variances of chromosomes *A*, *B*, and *C*, and in fact the joint analysis shows virtual identity of the two values. This result agrees with the observations of Bennett (1937) on *M* chromosomes in *Fritillaria chitralensis*.

These comparisons suggest, first, that the exact position at which pairing begins depends on the shape and size of the chromosome and, secondly, that a procentric habit may allow pairing to begin regularly on one side of the centromere. The question as to whether this second property is due to the low number of chromosomes and their easier movement in *Paris polyphylla* can be answered by comparison with the tetraploid *P. quadrifolia*.

4. MEIOSIS IN THE TETRAPLOID

Pairing in the tetraploid species (as in the octoploid, according to Haga, 1937) is entirely in bivalents. I have seen no quadrivalents. The tetraploid has an average chiasma frequency of 2·67 instead of the 3·25 of the diploid
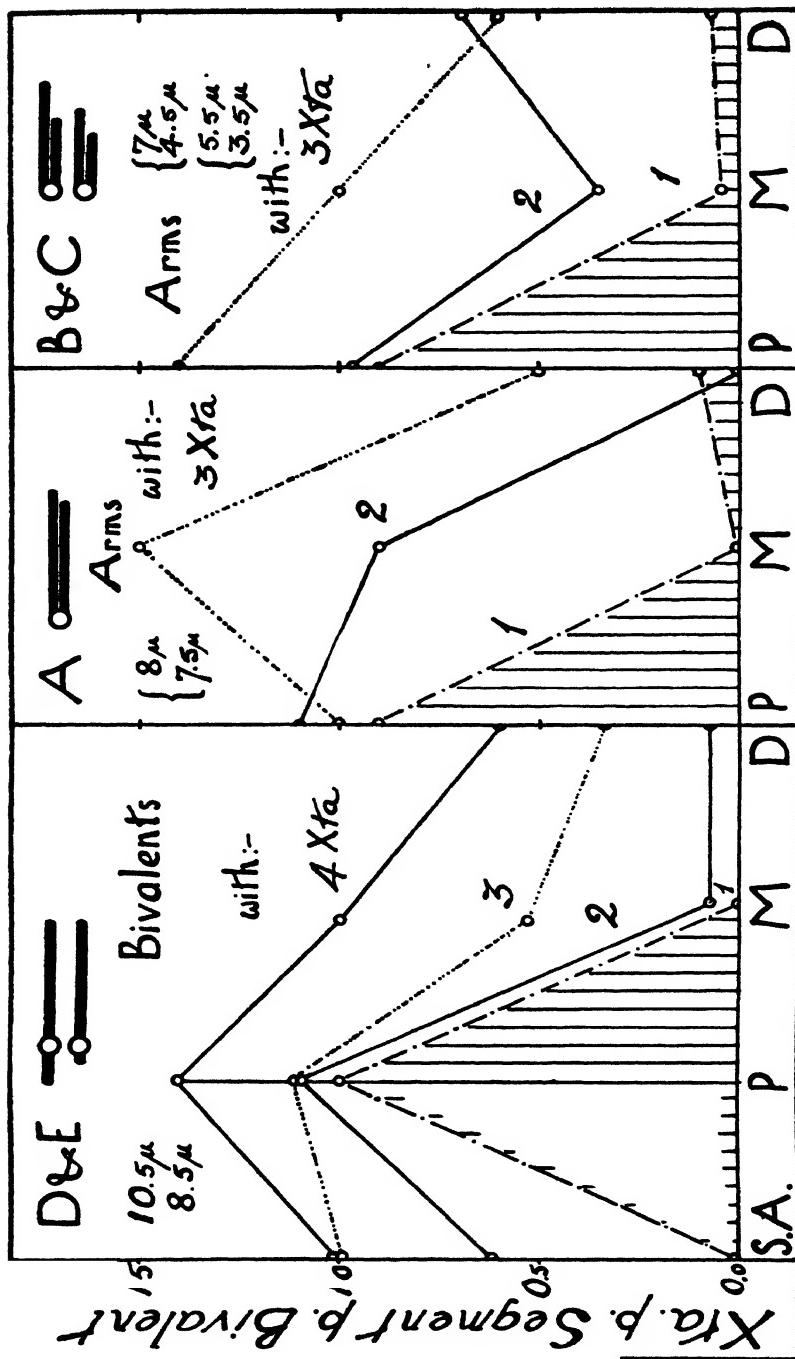
FIG. 8. Graph giving data of Table X, per bivalent: *P. quadrifolia*.

TABLE VIII

Paris quadrifolia, 10 Cells (cf. Table IV). Numbers of Chiasmata per Arm and per Whole Chromosome

Xta.	0.	1.	2.	3.	4.	5.	6.	Total.
Arms, A . .	5	21	12	2	—	—	—	40
Wholes „ . .	—	4	5	7	4	—	—	20
Arms, B . .	2	22	11	5	—	—	—	40
Wholes „ . .	—	—	8	4	8	—	—	20
Arms, C . .	2	23	12	2	1	—	—	40
Wholes „ . .	—	1	6	10	2	—	1	20
Wholes, DE .	—	5	15	15	5	—	—	40
Arms, ABC .	9	66	35	9	1	—	—	120
Wholes „ .	—	5	19	21	14	—	1	60

TABLE IX

Paris quadrifolia, 10 Cells (cf. Table V). Numbers of Chiasmata in Segments of Whole Bivalents

Chr.	S.	P.	M.	D.	Bivs.	Xta.	X/B.	Length. μ
A	—	34	14	3	20	51	2·6	18·5
B	—	40	9	10	20	59	3·0	12·5
C	—	33	10	14	20	57	2·9	9·0
DE	31	46	14	9	40	100	2·5	{ 11·5 / 9·5
Total	—	—	—	—	100	267	2·67	—

TABLE X

Paris quadrifolia (cf. Table VI)

DE bivalents.	S.	P.	M.	D.	T. bivs.	T. Xta.
1 X . .	—	5	—	—	5	5
2 Xta . .	11	17	1	1	15	30
3 Xta . .	15	17	8	5	15	45
4 Xta . .	5	7	5	3	5	20
A Arms					T. Arms	
1 X . .	—	19	0	2	21	21
2 Xta . .	—	13	11	—	12	24
3 Xta . .	—	2	3	1	2	6
BC Arms					T. Arms	
1 X . .	—	40	2	3	45	45
2 Xta . .	—	22	8	16	23	46
3 Xta . .	—	10	7	4	7	21
4 Xta . .	—	1	2	1	1	4

(Tables VIII and IX) and about 7 per cent. of its cells have unpaired chromosomes. The bivalents are comparable to those of the diploid in showing localization (Fig. 7). The reduction in chiasma frequency, however, necessarily entails a change in its distribution. How this happens is shown by

using the same method of analysis as in the diploid species (Table X and Fig. 8). Again, the separation of bivalents into classes according to the numbers of their chiasmata shows that the distribution of chiasmata changes as the number increases. Those with single chiasmata have them almost entirely in the proximal segment. Those with three or four chiasmata in one arm approach an even distribution.

Only two consistent differences distinguish the samples of the two species. First, there are fewer chiasmata in the middle parts of all the arms in the tetraploid species. The pachytene pairing must be more strictly localized. Secondly, the proximal chiasma is closer to the centromere in the tetraploid, so that two chiasmata can more commonly be concentrated in the proximal segment. Mather's differential distance (as in Fritillaria) is shorter. The pachytene torsion must be greater, so that the crossing-over per unit of length paired at pachytene can reach a higher concentration.

Thus two changes distinguish the tetraploid from the diploid, the one acting to reduce crossing-over, the other to increase it. The result is to leave it very much as it would have been with the operation of the reduction factor arising from the mere doubling of the chromosome number and the consequent hindrance to pairing. (Upcott, 1939; Darlington, 1940.)

5. THE CO-ORDINATION OF PAIRING

Certain peculiarities of meiosis do not appear in the small samples used for detailed analysis. Cells with unpaired chromosomes are particularly important in showing departures from the ordinary system of pairing, for in the same cells are usually found pairs with exceptional contact points. In *P. quadrifolia*, *D* and *E* chromosomes had single short-arm chiasmata and *A* chromosomes single middle-arm chiasmata. In *P. polyphylla* a group of cells showed the associated abnormalities of interlocking and failure of pairing. One of these cells even had an association of three, indicating a duplication. None of these things was found elsewhere. I have often noticed such a regional correlation of abnormalities in other species. It seems that complete failure of pairing of two chromosomes therefore arises from a disturbance of position in the chromosomes before pairing begins, and that this disturbance usually affects the whole nucleus. The same principle has been shown in a disturbed pollen mother cell of *Lilium candidum* and in an embryo sac mother cell of *L. Thunbergii* as well as in *Allium* hybrids (Darlington, 1940). It implies a regular orientation of the chromosomes in the leptotene stage of the normal nucleus as the basis of a regular co-ordination of pairing.

6. SUMMARY

1. Triploid forms of diploid species in Paris are presumed to be sterile, clonal, and autopolyploid. Tetraploid and octoploid species are sexually fertile and allopolyploid (Table I).

2. The five chromosome types are of similar shape in all species (Table II). In the tetraploid *P. quadrifolia* the chromosome and pollen-grain size is

reduced, in the octoploid where the pollen-grain size is proportionate the chromosome size is maintained (Table IV).

3. The diploid *P. polyphylla* has, like Trillium species, no nucleolar organizers and over 10 per cent. of heterochromatin, i.e. of genes showing allocycly (Darlington and La Cour, 1938).

4. Other diploid species have one nucleolar organizer in each haploid set and it is to be expected that they will show no allocycly.

5. *P. quadrifolia* has no allocycly and, although tetraploid, it forms no quadrivalents at meiosis and has only one nucleolar organizer in its. two haploid sets (cf. *Hyacinthus orientalis*).

6. The allocycly of *P. polyphylla* is confined to the distal segments with least crossing-over, but the same proximal localization of crossing-over is found in all species of Paris without regard to polyploidy or allocycly. Chiasma analysis of bivalents shows the same procentric order of pairing in diploid and tetraploid (Figs. 6 and 8).

7. This comparison suggests that (i) the same genes which show allocycly in one species do not do so in another, i.e. allocycly and perhaps inertness are genotypically controlled, and (ii) this control is related to the activity of the nucleolar organizers.

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Studies in Tropical Fruits

XI. Carbohydrate Metabolism of the Banana Fruit during Ripening under Tropical Conditions

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With thirteen Figures in the Text

I. INTRODUCTION

AN account of the changes in the carbohydrate metabolism during the growth and ripening of the Gros Michel banana fruit on the plant has already been published (Barnell, 1940). Such a study provides a survey throughout

development of material whose behaviour in storage is to be investigated. The natural sequel—the investigation, under tropical conditions, of ripening processes in bunches cut at the several stages of maturity adopted by the trade —is described in the present paper. The considerable amount of analytical work involved has made it necessary to limit this inquiry at present to Gros Michel fruit harvested at one particular level of development, that described as 'heavy ¾-full'[1]; fruit so harvested, when ripened under tropical conditions of fluctuating temperature and humidity, exhibits the nearest approximation to 'normal' ripening.[2] Concurrently with this work, fruit cut '¾-full' and 'heavy ¾-full' was sampled while undergoing various storage treatments, so that comparisons might be made of the carbohydrate changes occurring under the several conditions; the results of these studies will be described later.

The unripe banana fruit contains a large amount of stored carbohydrate in the form of starch and a small amount of sugar, including sucrose, glucose, and fructose. It is known from other workers' results, e.g. Bourdouil (1931), Stratton and von Loesecke (1930), that ripening is accompanied by a reduction to small proportions of the amount of starch, with a coincident rise in total sugar. The interrelations of sucrose and hexose sugars and the behaviour of the individual sugars during ripening, as determined by the several investigators, show, however, a considerable lack of agreement. This is not unexpected in that fruit of several varieties and from very different localities was used, and, in addition, was subjected to different storage treatments before ripening. Thus early and considerable increase in the sucrose concentration is a feature of the ripening studies of Canary bananas made by Bourdouil (1931) and of Gros Michel by Poland, Manion, Brenner, and Harris (1938); but while in the former study hexose sugars continue to increase in concentration while sucrose falls, in the latter sucrose and hexose concentrations fall simultaneously. Stratton and von Loesecke (1930) found in Gros Michel that both sucrose and hexose sugars increased in concentration at approximately the same pace in the early stages of ripening, but that sucrose soon reached a peak value while hexose sugars continued thereafter to increase considerably.

In the present work the observations made on the several carbohydrate metabolites during ripening under tropical conditions have been expressed (i) as percentages of the fresh weight at sampling of the pulp or skin which were analysed separately, and (ii) as the amount present in the pulp or the skin of a single fruit. From (ii) the drift of the total estimated carbohydrate in pulp and skin may be found, and, if the assumption be made that none of it has been derived from the main stalk of the bunch, or transformed into other substances not estimated in these studies, then the rate of loss of

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[1] This is the grade of fruit harvested in Trinidad for the Canadian market and described in a previous communication (Wardlaw, Leonard, and Barnell, 1939) as '90' day fruit, weighing 150–60 gm. per finger.

[2] Since the Gros Michel banana does not ripen in a state of nature while attached to the tree and in the absence of disease or injurious agents, the term 'normal' ripening requires qualifying.

total carbohydrate may be regarded as giving the rate of loss due to respiratory processes. Figures based on this assumption are presented in section IX and comparison made with the total loss of dry matter and with the data available on CO_2 liberated. Since cellulose, hemi-celluloses, and other non-estimated carbohydrates or carbohydrate sources may provide respirable materials, the assessment of respiration rates on the basis of ascertained carbohydrate trends may be invalid. The procedure, however, has definite analytical value in that it may afford some indication of the part which certain metabolites may play in the complex process of respiration. Provided there is no interchange of substances between finger and bunch stalk it is evident that the loss of materials due to respiration may be properly deduced from the rate of loss of total dry matter in each tissue. Relevant data have accordingly been put forward.

II. Materials, Methods and Procedure

The fruit used in the present investigation was selected as 'heavy ¾-full', obtained from the Toco district of Trinidad. The fruit, wrapped in banana trash to reduce damage in handling, was delivered at the Research Station within six or seven hours of cutting; thirty bunches were selected, numbered, and stood in line against wooden supports in the laboratory, and hence were subject to fluctuating temperature and humidity. The first sample was taken as soon as possible after arrival, two more within the first twenty-four hours, and thereafter at two-day intervals until the 11th day, a final sample being taken on the 15th day.

Each sample consisted of thirty fingers, one finger from each bunch taken from the middle fingers of the third hand (Wardlaw, Leonard, and Barnell, 1939). The thirty fingers were weighed together and then separated into skin and pulp which were sliced into small pieces and weighed. The pulp and skin in separate trays were placed in a freezing cabinet at approximately $-20°$ F. ($-30°$ C.) and left to freeze over-night. In this manner all the processes of the living tissues were quickly slowed down and the tissue rendered suitable for maceration and subsampling for subsequent analysis. The samples were wrapped in canvas cloths and pounded with a heavy mallet in the freezing-room at a temperature of $0°$ to $10°$ F. The pulp of unripe fruits and the skin at all stages gave a powder which was sieved to give a fine homogeneous medium from which representative samples of 30 gm. each in duplicate were weighed out and placed immediately in soxhlet thimbles. The pulp of ripe fruit gave a paste on pounding; this was mixed and made as homogeneous as possible by vigorous rolling with a rolling pin on a pastry board; the paste was sampled in 30 gm. lots direct into soxhlet thimbles. The pulps of fruits of intermediate age gave little trouble: hard pounding of the frozen tissue yielded a very stiff paste which, with mixing and rolling, became reasonably uniform for sampling.

The samples in the soxhlet thimbles were extracted for six hours with

alcohol (approximately 80 per cent.), the extracted residues being placed in the alcohol extract in sealed glass jars and stored to await analysis.

For subsequent analysis the residue in the thimbles, together with any solid matter in the extract, was separated from the extract by filtration through hard filter-papers and the residues washed copiously with warm alcohol. The extracted residue was dried at 100° C. and weighed; samples from it were used for the estimation of the starch content. The alcohol extract was made up to volume and aliquots used to determine titratable acid[1] and total alcohol-soluble substances. The further treatment of the alcohol extract and the methods of estimation of the various carbohydrates remained the same as described in previous communications (Barnell, 1936, 1938, and 1940).

All the carbohydrate quantities given are in terms of glucose.

III. Changes in Weight of Whole Fingers, Pulp, and Skin during Ripening

Fruit ripened at the high temperature of the laboratory (70°–85° F.) falls in weight due to water and respiratory loss, but not at a uniform rate. The rate of loss in weight of bananas as fingers, hands, and bunches at constant high temperatures and humidities has been discussed by Leonard (1941). The rate of water loss is dependent on various external factors, on the physico-chemical properties of the pulp and skin components, and on the structure of the skin and pulp. During ripening considerable changes occur in the nature of the constituents of both pulp and skin, and associated with them are changes in their water-retaining forces. Hence the varying rates of water loss observed.

Fig. 1 shows the mean fresh weights at sampling of single fingers, pulps, and skins plotted against time for bananas ripening on the bunch. The whole finger lost weight, mainly through water loss, throughout the ripening period, but more rapidly during the 2nd and 3rd days than during the 1st or the 4th to 9th days. The rate of loss between the 9th and 11th days, when the fruit was changing from the ripe to the over-ripe condition, was the most rapid of the whole period.

The pulp and skin lost approximately equal amounts of water during the 2nd and 3rd days (Fig. 1), the skin having a rate of loss nearly 50 per cent. above that of the pulp. After the 3rd day the curves for skin and pulp diverge; the pulp increased in weight while the skin continued to lose weight more and more rapidly, culminating in a loss of over 13 gm. between the 9th and 11th days, after which only 1 gm. was lost between the 11th and 15th days.

Concerning the 2nd and 3rd day little more can be said at present than that this was the time when the fruit attained the 'sprung' condition, i.e. began

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[1] In several instances an excess of calcium carbonate was added to the alcohol used for extraction to neutralize the acid extracted from the tissue and so prevent sucrose inversion, but comparison with similar extracts to which no calcium carbonate was added showed that inversion due to extracted acid, if any, was negligible.

to yield to the touch and to change colour slightly. This occurs shortly after the climacteric respiration peak (Wardlaw and Leonard, 1940), when respiration rate, internal concentration of carbon dioxide, and carbon dioxide content of tissue begin to decline and internal oxygen concentration increases.
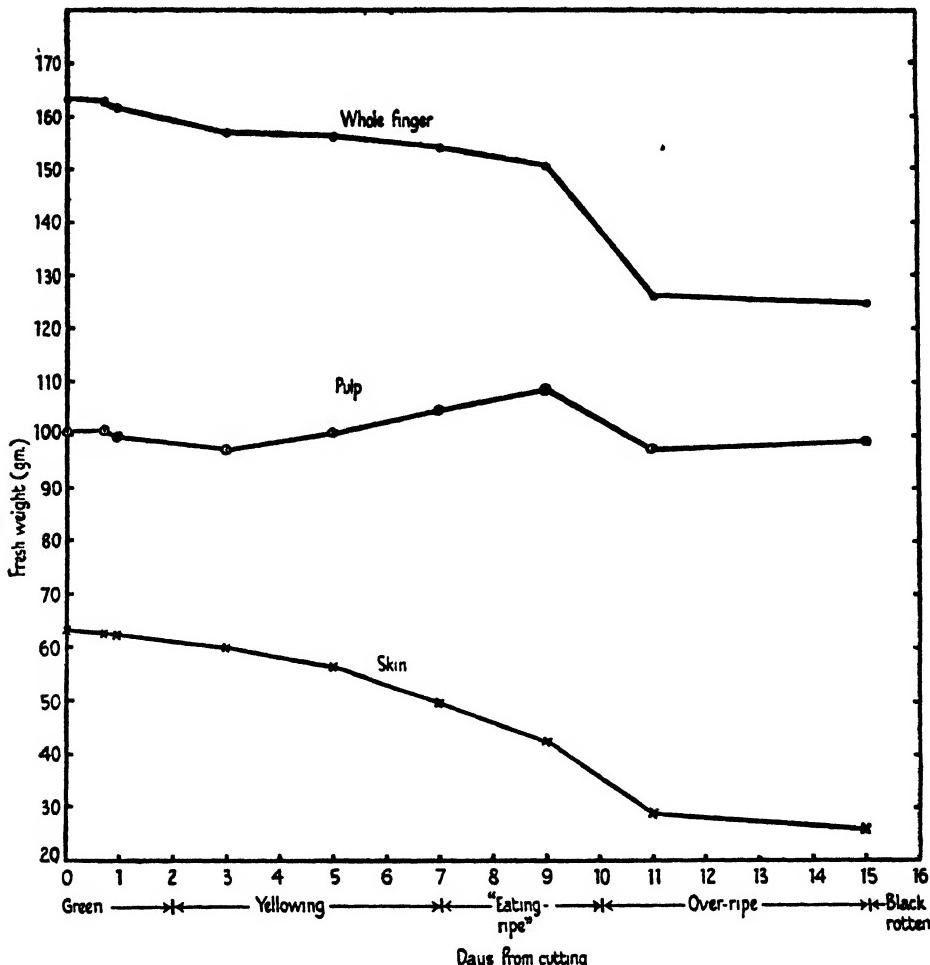
FIG. 1. The mean fresh weights of pulp, skin, and whole finger of 'heavy ⅓-full' bananas during ripening. Each value is the mean weight per finger obtained from the weight of thirty fingers, each taken from the third hand of a bunch. Increasing weights are shown by the pulp tissues during the yellowing and 'eating-ripe' stages: during the over-ripe stage the whole finger and both skin and pulp lost weight.

Together with these changes the increased rate of water loss may be ascribed to the organization changes induced by the conditions resulting from the severance of the bunch from the plant; in addition the relatively high tissue temperatures (about 1° F. above the surrounding air) may account for part of the increased rate of water loss.

The increase in weight of the pulp which followed the 3rd day was co-incident with a rapid increase in the concentration of sugars (Fig. 2). The suction pressures of the pulp cells become sufficiently high to cause the with-
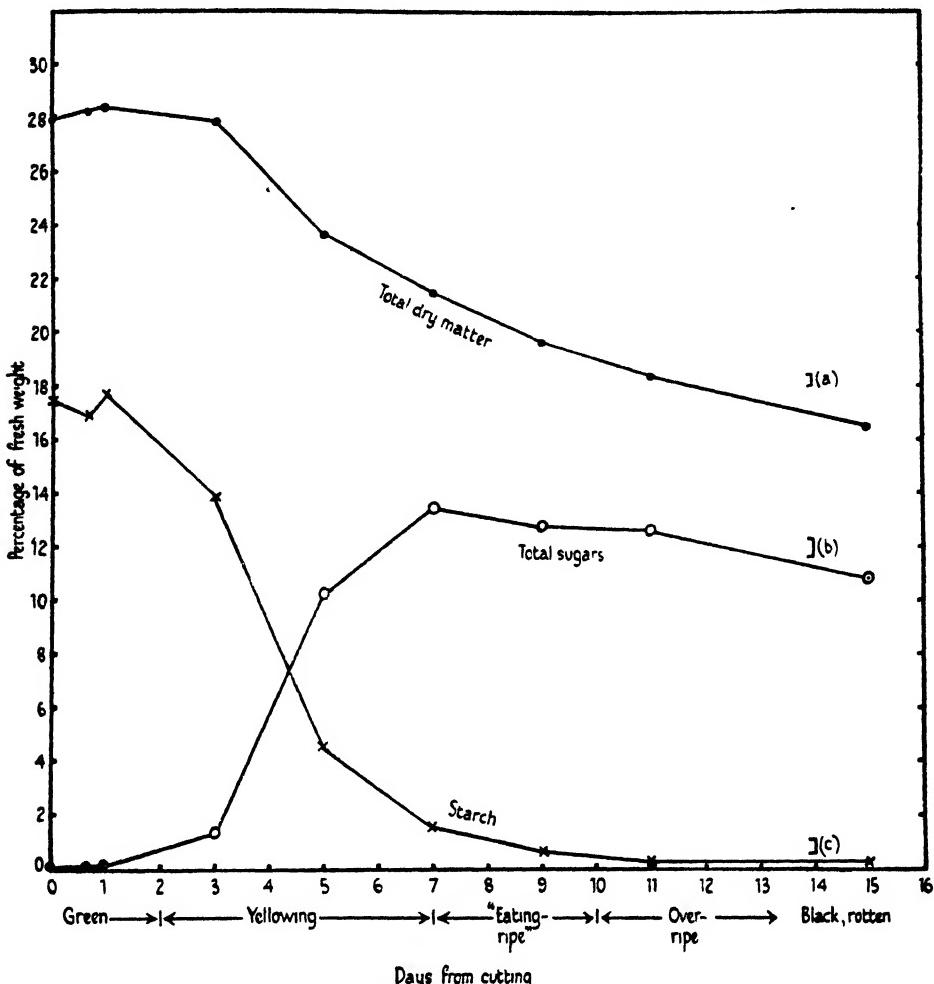
FIG. 2. Pulp. Dry matter, starch, and total sugars as percentages of the fresh weight during ripening under tropical conditions. The vertical lines labelled (*a*), (*b*), and (*c*) represent the minimum significant difference ($P = 0.05$) of (*a*) dry matter, (*b*) total sugars, and (*c*) starch.

drawal of water from the bunch stem and skin tissue. After the 9th day a further change in the pulp cells appeared to take place which reduced the effective suction pressures exerted by the high sugar concentrations in these cells, water loss occurring between the 9th and 11th days. There was little change between the 11th and 15th days, a steady state having presumably been reached between loss of water by transpiration and water uptake.

From the 3rd day onwards the skins lost water more and more rapidly but

most rapidly between the 9th and 11th days, suggesting that this period was one of considerable organization change in the skin as well as in the pulp. The loss between the 11th and 15th days was small.

Although the water-absorbing ability of the skin must have increased after the 3rd day as a result of its rising sugar concentration (Fig. 3) enabling it to withdraw water from the bunch stem, it nevertheless lost water rapidly to the pulp because of the much greater increase in sugar concentrations in that tissue (Fig. 2).

IV. Percentage Amounts of Dry Matter and Various Carbohydrates in the Pulp

(a) *Total dry matter, starch, and total sugars.*

The changes during ripening in the dry matter of the banana pulp and of the two major carbohydrate components, starch and sugar, are shown as percentages of the fresh weight in Fig. 2. The percentage of dry matter increased slightly during the first twenty-four hours, but during the 2nd and 3rd days, when a high respiration rate (see section IX) accompanied the water loss, the net result was a reduction in the percentage dry matter content. During the succeeding days, from the 3rd to the 9th the percentage dry matter fell as the pulp absorbed water; the fall was very steep between the 3rd and 5th days. The fall continued to the end of the experiment when the fruit was black and rotten.

The starch content of the pulp began to fall on the 2nd day, fell rapidly to the 5th day, then less rapidly to the 9th day, by which time the content of true starch in the pulp was negligible.[1] A small part of the taka-diastase hydrolysis product had reducing properties, but was not glucose since it was not destroyed by a yeast capable of fermenting glucose, fructose, and sucrose.

Total sugars (sucrose, glucose, and fructose) had a clearly defined drift, shown in Fig. 2. The percentage increased slowly during the first twenty-four hours, more rapidly during the next two days and after the 3rd day very rapidly, attaining a peak value on the 7th day; thereafter it slowly declined. The attainment of the peak value coincided with the beginning of the stage indicated as 'eating-ripe'; this was immediately preceded by the yellowing stage which followed on the condition known as 'sprung'.

(b) *Sucrose, glucose, fructose, and glycosidic-glucose.*

The percentage amounts of the three individual sugars, sucrose, glucose, and fructose and of glycosidic-glucose in the pulp are set out in Fig. 4.

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[1] Fermentations of the taka-diastase hydrolysis products of the pulp were carried out on the first three samples during the present investigation and the residual reducing values estimated; the taka-diastase hydrolysis product values and the non-fermentable fraction values both expressed as glucose per 100 gm. fresh weight were: (1) 17·45, 0·67; (2) 16·90, 0·61; (3) 17·65, 0·61. Thus if part or most of the total hydrolysis products on and after the 9th day of ripening consists of the non-fermentable fraction the fraction derived from true starch must be very low.

During the 1st day after cutting there was little change, but this was followed by a considerable increase in all the three sugars during the 2nd and 3rd days when fruits attained to the 'sprung' condition and yellowing began. Up to
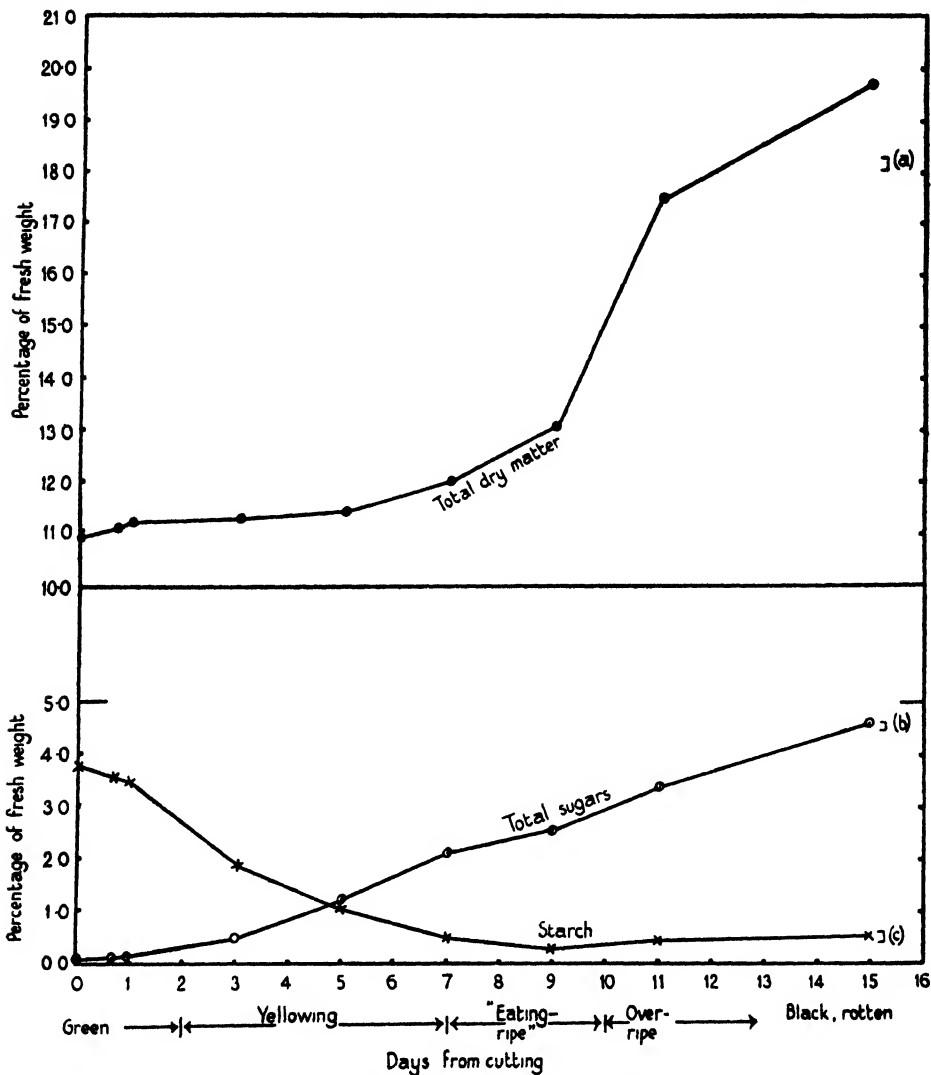
FIG. 3. Skin. Dry matter, starch, and total sugars as percentages of the fresh weight during ripening under tropical conditions. The vertical lines labelled (*a*), (*b*), and (*c*) represent the minimum significant difference ($P = 0.05$) of (*a*) dry matter, (*b*) total sugars, and (*c*) starch. The base line for the dry matter observations has been drawn at an ordinate value of 10 per cent.

the 3rd day sucrose rose more rapidly than the hexose sugars, but by the 5th day the percentage of glucose was slightly greater than that of sucrose, whereas fructose was still present in less amount. Sucrose increased only

slightly to the 7th day, afterwards falling in amount during the 'eating-ripe' stage and then remaining at 1 per cent. during the late senescent stage of the 11th to 15th days.

Glucose and fructose continued to increase during the 'eating-ripe' stage,
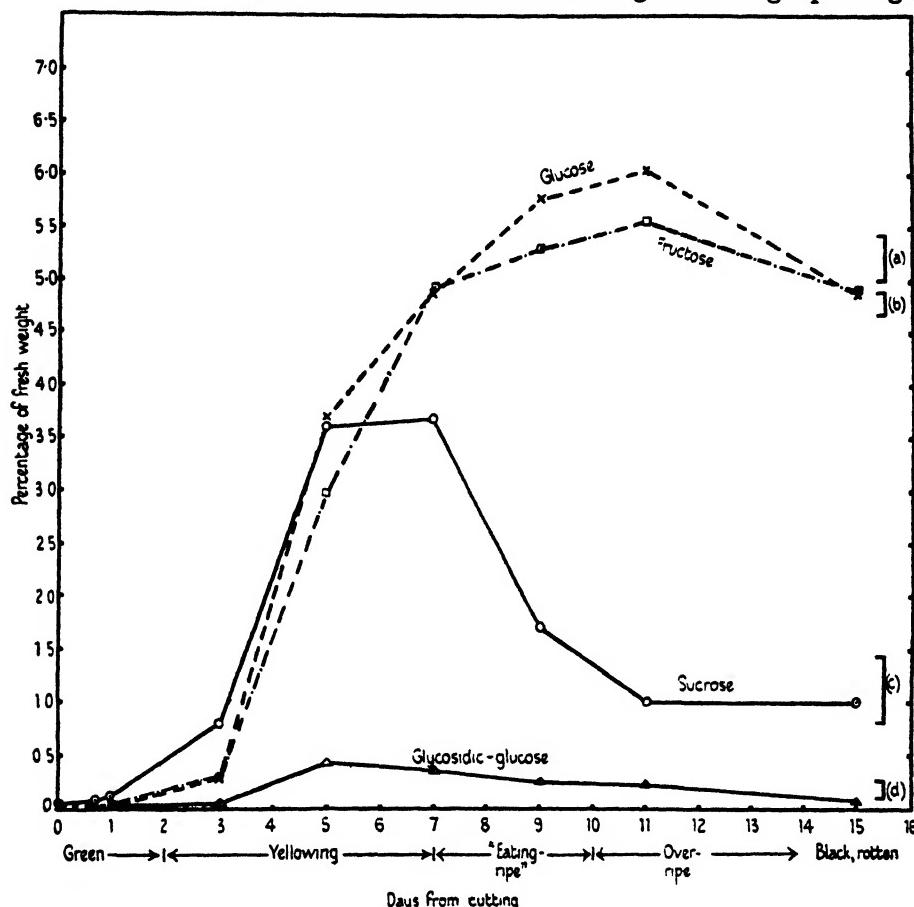
FIG. 4. Pulp. Sucrose, glucose, fructose, and glycosidic-glucose as percentages of the fresh weight during ripening of 'heavy ¾-full' grade fruit under tropical conditions. The vertical lines labelled (a), (b), (c), and (d) represent the minimum significant differences of (a) glucose, (b) fructose, (c) sucrose, and (d) glycosidic-glucose.

both attaining peak values on the 11th day when the fruit was over-ripe and anthracnose spots were developing on the skin. Glucose was present in higher concentration at this stage, but the concentrations of glucose and fructose were approximately the same[1] on the 15th day.

[1] The approximate equality of the glucose and fructose quantities, together with the relatively low sucrose values during the 'eating-ripe' stage, suggested that inversion of cane-sugar might have occurred during the storage of the samples while awaiting analysis. But analysis of duplicates, some of which had been retained in storage longer than the others by

Glycosidic-glucose increased to the 5th day, then declined slowly throughout the ripe and late senescent stages.

(c) *Titratable acid.*

The values for titratable acidity, given in column 2 of Table I, did not permit of a high degree of accuracy since the actual titration readings were very small (of the order of 0·2 c.c.).

The initial increase between 0 and 16 hours was not significant, so that, in general terms, the drift followed a downward course as the fruit approached the sprung condition and then, during the stage of advancing yellowing, the acidity rose, attaining its maximum value at the beginning of the 'eating-ripe' stage and maintaining this value through most of that stage, but afterwards falling during late senescence as the skin turned brown-black and the pulp became more and more liquid.

It is possible that the rising acid values observed during the ripening of the pulp are connected with incomplete oxidation of carbohydrates during respiration, when internal oxygen concentrations fall to very low values (Wardlaw and Leonard, 1940). Correlated respiration studies, including respiratory quotient measurements, are required together with more detailed acid observations.

V. Percentage Amounts of Dry Matter and Various Carbohydrates in the Skin

The estimation separately of the dry matter and carbohydrates of the skin and pulp of the ripening banana permits of the consideration of the biochemical interrelations of these components. The banana fingers were ripened on the bunch so cannot be considered as independent units but, so far as can be judged from the data presented in this paper, there is little interchange either between the main stem and the fingers of the bunch or between the different fingers (see Appendix, Tables V and VI, for amounts of dry matter in pulp and skin). Accordingly a study of the biochemistry of pulp and skin during ripening gives a picture of the changes proceeding in each and of the effects produced by changes in one organographic region on the composition of the other. Thus changes in the carbohydrate composition of the pulp are particularly noticeable in the effect they produce on the percentage of dry matter in the skin.

(a) *Total dry matter, starch, and total sugars.*

The trend of dry matter in the skin as shown in Fig. 3 was practically the converse of that in the pulp (Fig. 2). The percentage amount increased all

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several weeks, gave no indication of inversion having occurred during the interval. Also, as stated before (p. 220), the neutralization of the acid in the tissues by the addition of excess of calcium carbonate with the boiling alcohol had no effect on the subsequent analysis.

through the period investigated, but rose particularly rapidly during the 'eating-ripe' and over-ripe stages. From this it may be concluded that as the sugars increased in the pulp there was an increasing loss of water to the pulp until the over-ripe stage; thereafter, changes in the structure of the skin permitted greatly increased transpiration losses, chiefly from the skin, as denoted by the rapidly rising percentage dry matter content (Fig. 3) during that period. Water uptake from the skin by the pulp decreased in the over-ripe stage as indicated by a less rapid decrease in percentage dry matter content (Fig. 2). Since there was a loss of total weight of the pulp during the over-ripe stage (Fig. 1) a relatively high rate of respiration, probably anaerobic, may be inferred, thus producing the falling values for dry matter content shown in Fig. 1, which must otherwise have shown rising values due to the water loss (cf. section IX).

The starch content of the skin fell simultaneously with that of the pulp (cf. Figs. 2 and 3), a low value for the total taka-diastase hydrolysable substance being obtained on the 7th day, representing a negligible starch content.[1] There was a further fall to day 9 followed by a slight rise, not statistically significant but consistent over the two sampling dates (days 11 and 15), possibly due to the presence of increasing amounts of hemicelluloses or other metabolites also subject to hydrolysis by taka-diastase.

Total sugars in the skin increased slightly during the 1st day (Fig. 3), more rapidly during the 2nd and 3rd days, and then steadily throughout the entire period to the last sampling date, when the fruit was black and rotten.

(b) *Sucrose, glucose, fructose, and glycosidic-glucose.*

The drifts during ripening of the percentage amounts in the skin of each of the sugars are plotted in Fig. 5. The percentage amounts of all the sugars were much lower than in the pulp throughout the ripening process. Sucrose and glucose were present in approximately equal concentrations during the 1st day, but sucrose rapidly increased as yellowing began in the skins. After the 5th day the rise in concentration was arrested but continued again from the 7th to the 11th day, with little subsequent change to the last sample on the 15th day.

Glucose and fructose increased at first slowly and then after the 3rd day rapidly and consistently until the last sampling date, glucose being always in excess of fructose. A clearer picture of the changes in actual amounts of the sugars during the later phases of ripening is given in section VII, Fig. 9, where total amounts per finger are considered, the effect of the superimposed increase in dry matter (i.e. decrease in water content) being thereby removed.

Glycosidic-glucose increased consistently in the skins during ripening until the 11th day, when the fruit was over-ripe; it then fell slightly to the 15th day.

[1] See footnote to section II.
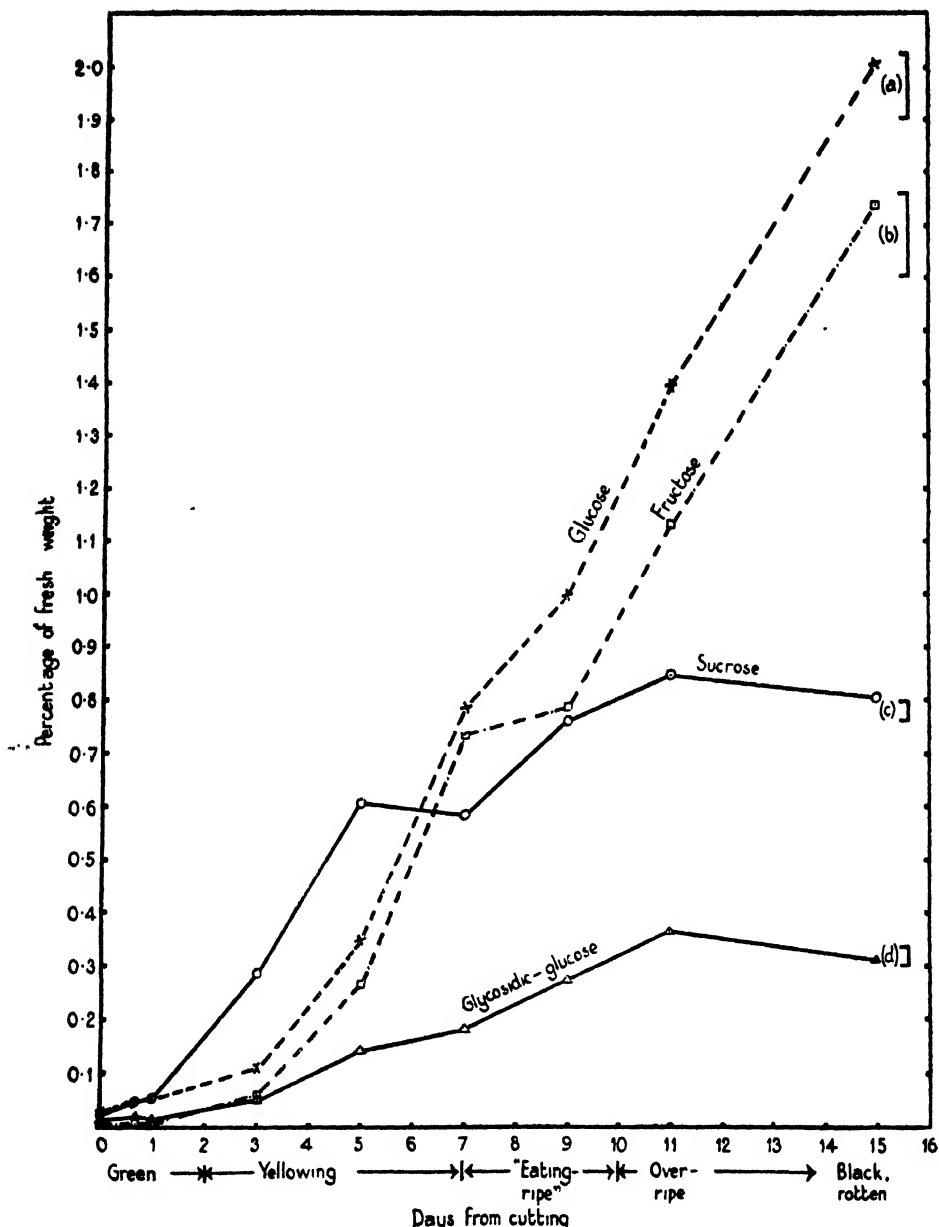
FIG. 5. Skin. Sucrose, glucose, fructose, and glycosidic-glucose as percentages of the fresh weight during ripening of 'heavy ½-full' grade fruit under tropical conditions. The vertical lines labelled (a), (b), (c), and (d) represent the minimum significant differences of (a) glucose, (b) fructose, (c) sucrose, and (d) glycosidic-glucose.

(c) *Titratable acid.*

The drift of titratable acid in the skin shown by the values in column 3 of Table I is, in general, similar to that of the values for the pulp (column 2), but with a time difference. The initial decrease shown is not significant and the
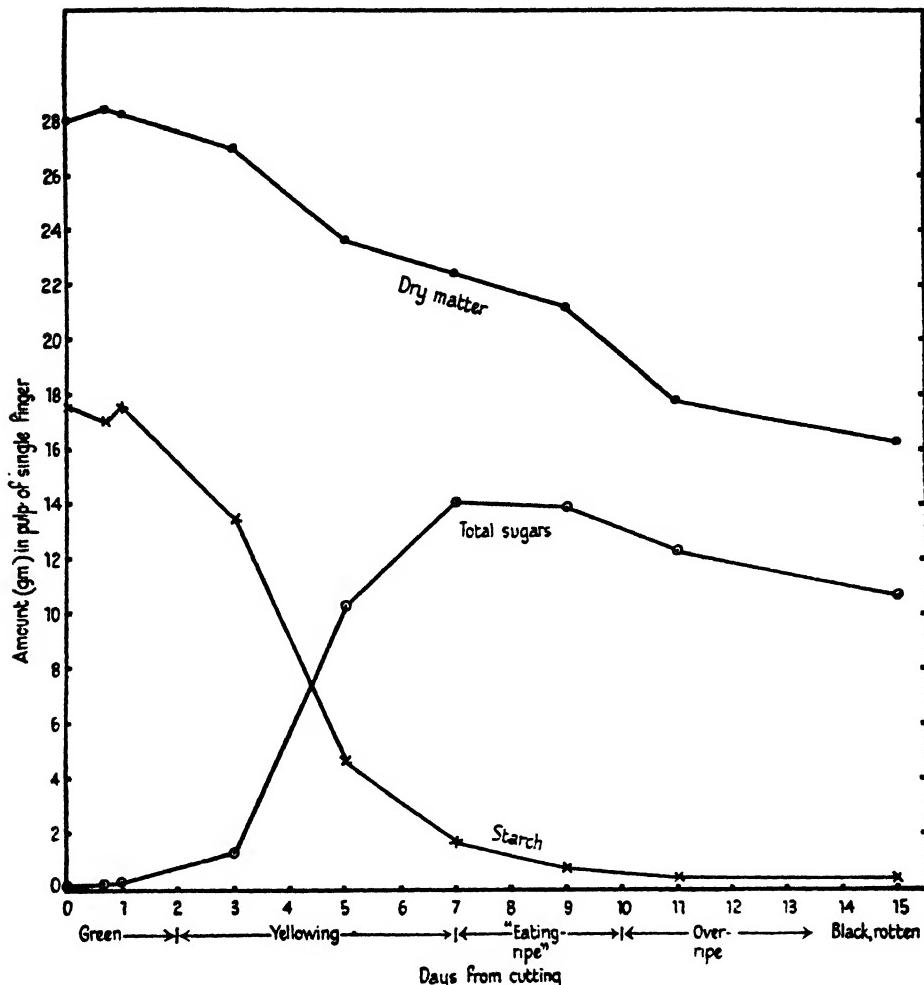
FIG. 6. Pulp. Amounts (gm.) of dry matter, starch, and of total sugars present in pulp of single finger at the various stages of ripening of 'heavy ⅓-full' fruit stored under tropical conditions.

increase in acidity associated with ripening, though possibly beginning after the 3rd day, only became clearly evident after the 5th day. This continued through the yellowing and ripe stages into the over-ripe stage on the 11th day, after which a decrease took place to the 15th day, when the fruit was black and rotting. In the pulp the increase began definitely after the 3rd day and decreasing values supervened after the 9th day, so that on the basis of these

acid values the pulp was approximately two days ahead of the skin in its ripening sequence. The possible connexion in the pulp between changes in acidity and respiratory phenomena (discussed in IV (*c*) above) may also apply to the values obtained for the skin during the ripening of the fruit.

Later work has shown that the skin tissue, in contrast to the pulp, is highly

TABLE I

Titratable Acid

(1) Time from cutting.	(2) Acid in pulp (ml. N/10 NaOH per 100 gm. Fr. Wt.).	(3) Acid in skin (ml. N/10 NaOH per 100 gm. Fr. Wt.).	(4) Acid in pulp of single finger (ml. N/10 NaOH).	(5) Acid in skin of single finger (ml. N/10 NaOH).
0	43·7	37·8	43·9	24·4
16 hrs.	53·2	32·8	53·5	20·5
23 ,,	52·4	32·8	52·1	20·4
3 days	38·2	32·0	37·0	19·1
5 ,,	54·4	33·0	54·6	18·5
7 ,,	65·0	39·1	68·0	19·3
9 ,,	64·9	57·9	70·3	24·5
11 ,,	51·7	64·2	50·3	18·5
15 ,,	43·9	56·9	43·5	14·8
Sig. diff. (*P* = 0·05)	13·7	6·72	—	—

buffered; increasing titratable acidity being accompanied by rising pH values. The implications of this and its possible relation to the relatively high carbon dioxide content found in late senescent skin tissue (Wardlaw and Leonard, 1940) will be reserved for discussion in a later communication.

VI. PERCENTAGE AMOUNTS OF TOTAL ALCOHOL-SOLUBLE SUBSTANCES, SUGARS, AND NON-SUGARS IN PULP AND SKIN

The possible importance of the non-sugar component of the total substances soluble in alcohol has been discussed in a previous communication (Barnell, 1940). In the present work no separate estimations were made of the constituents of the non-sugar component, fats, tannins, nitrogenous substances, &c., apart from the measurements of titratable acid and of glycosidic-glucose, but values for the total alcohol-soluble substances, total sugars (sucrose+glucose +fructose), and total non-sugars (by difference), for both pulp and skin, are set out in Table II.

In both pulp and skin of the freshly cut green banana the alcohol-soluble substances consisted to a very large extent of the non-sugar component (values for 0, 16 hrs., 23 hrs., in Table II), but while the sugars increased in concentration in both pulp and skin during ripening the proportion of non-sugars to sugars decreased. The substances included in the non-sugar component are therefore clearly of considerable importance in the unripe
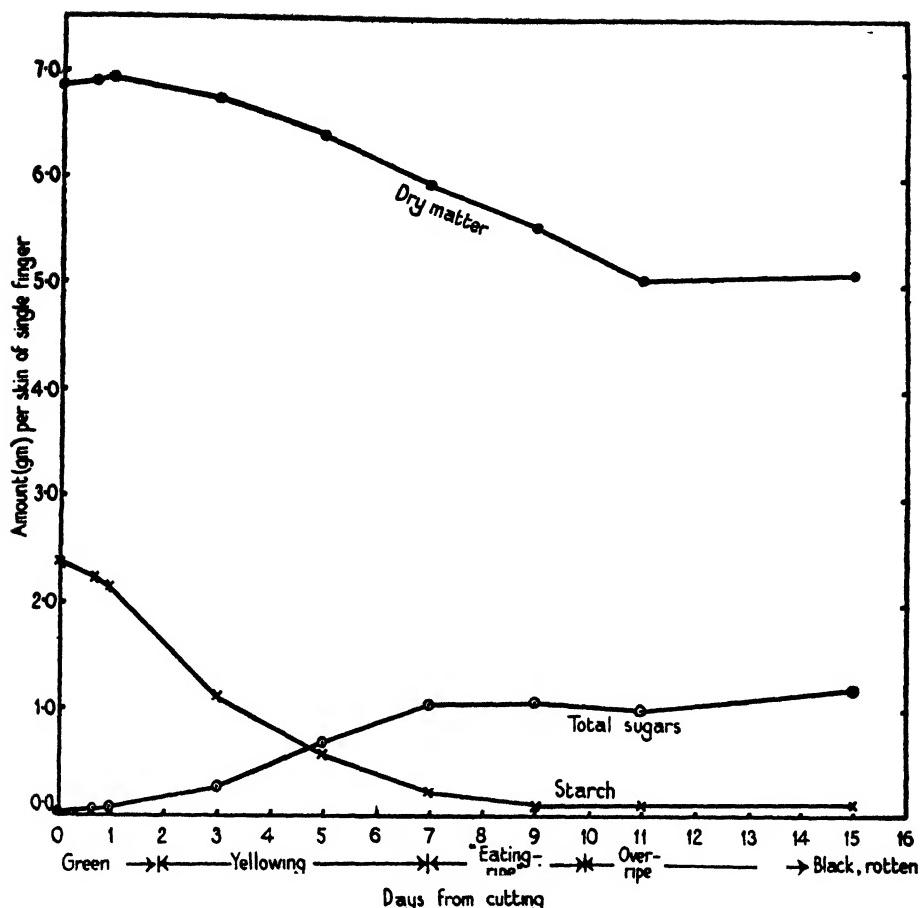
FIG. 7. Skin. Amounts (gm.) of dry matter, starch, and of total sugars in skin of single finger at the various stages of ripening of 'heavy ¾-full' fruit stored under tropical conditions.

TABLE II

Total Alcohol-soluble Substances, Total Sugars and Non-Sugars in Pulp and Skin (Expressed as percentages of the Fresh Weight)

(1)	(2)	(3) Pulp.	(4)	(5)	(6) Skin.	(7)
Time from cutting.	Total alcohol-soluble substances.	Total sugars.	Non-sugar (by difference).	Total alcohol-soluble substances.	Total sugars.	Non-sugar (by difference).
0	1·15	0·097	1·05	0·95	0·057	0·89
16 hrs.	1·76	0·125	1·63	1·16	0·093	1·07
23 ,,	1·53	0·182	1·35	1·08	0·110	0·97
3 days	2·17	1·315	0·85	2·03	0·453	1·58
5 ,,	13·22	10·225	2·99	3·28	1·219	2·06
7 ,,	15·89	13·450	2·44	4·08	2·105	1·97
9 ,,	15·86	12·770	3·09	4·33	2·521	1·81
11 ,,	15·38	12·600	2·78	5·66	3·379	2·28
15 ,,	13·47	10·770	2·70	6·91	4·600	2·31

banana, as judged by their magnitude; the drift of their percentage amounts during ripening suggests that though they may become quantitatively less important in relation to sugars they yet play some very definite role in the metabolism of ripening in both pulp and skin.

There are some inconsistencies in the drifts of the non-sugar component in pulp and skin (columns 4 and 7 of Table II) which perhaps suggest that the extraction of this fraction by alcohol may not be quantitative.

VII. Changes in Total Amounts per Finger of Dry Matter and Various Carbohydrates in the Pulp

The large drifts in the water contents of the pulp and skin of the banana during ripening render it desirable that quantitative data for other constituents should be presented in a manner which eliminates the arithmetical effects of these changes of water content. This is best achieved by giving the actual composition of the fruit as the total amounts of each estimated substance present per finger in the pulp and in the skins. Since the samples were moderately large, 30 fingers to each sample, the mean weight per finger at each stage of ripening is a satisfactory statistic for the population, especially as samples were taken from the same hand (the third) in each bunch.[1] In the present section, therefore, the data are presented as amounts in the pulp of a single finger and in the next section as amounts in the skin of a single finger.

(a) *Total dry matter, starch, and total sugars.*

Fig. 6 shows, on this basis, the changes which occurred in the amounts of dry matter, starch, and total sugars in the pulp during ripening. The dry matter content of the pulp fell throughout the entire period of ripening and late senescence, while the starch fell in amount as the sugars simultaneously increased. But the amount of total sugars formed did not attain the value of the original starch content of the pulp, the difference being due, in part at least, to loss of carbohydrates in respiration. This carbohydrate loss by respiration will be discussed in some detail in the section on utilization rates in pulp and skin (section IX).

A closer examination of the dry matter curve shows that the rate of decrease was not uniform; there were two periods of more rapid loss than that of the general drift, one during the interval between the 3rd and 5th days and the other between the 9th and 11th days. The first phase of more rapid loss was coincident with the high rate of increase of soluble sugars in the pulp (Fig. 7) and of water uptake (Fig. 1), and also with the attainment of the 'sprung' condition which is accompanied by a high rate of respiration. The second phase occurred as the fruit passed through the 'eating-ripe' to the over-ripe stage when the tissues, both pulp and skin, lost water rapidly (Fig. 1) and are known to have a high respiration rate; the carbon dioxide

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[1] The marked uniformity between the individual fingers in a hand has been noted previously (Wardlaw, Leonard, and Barnell, 1939).

formed being partly liberated and partly accumulated within the tissues (cf. Wardlaw, Leonard, and Barnell, 1939 *a*, Fig. 6; also Wardlaw and Leonard, 1940).

The curves for starch and total sugar are similar in general form to those
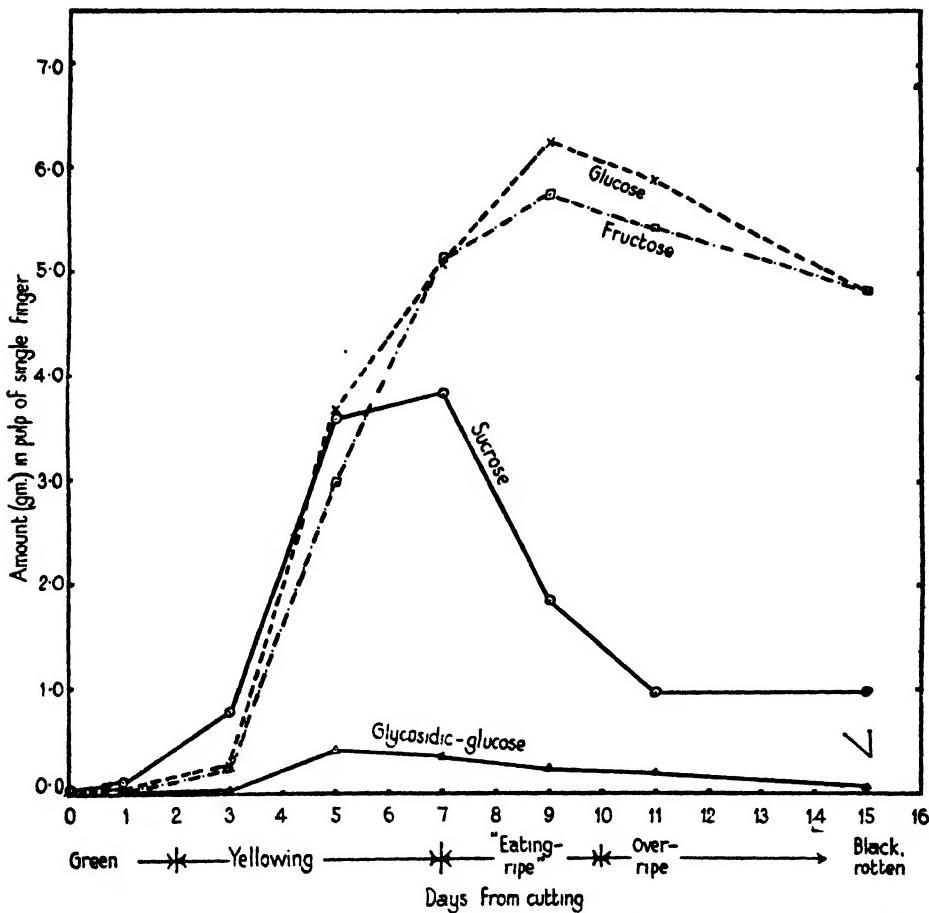
FIG. 8. Pulp. Amounts (gm.) of sucrose, glucose, fructose, and of glycosidic-glucose in pulp of single finger at the various stages of ripening of 'heavy ¾-full' fruit stored under tropical conditions.

for percentage amounts shown in Fig. 2. Total sugars increased until the 7th day and then remained at the maximal value till the 9th day; after this no further supplies of sugar were available from starch hydrolysis. Total sugars fell uniformly from the 9th day to the last sampling date on the 15th day, being consumed in the respiratory process or possibly transformed into other compounds.

(b) *Sucrose, glucose, fructose, and glycosidic-glucose.*

The amounts of each of the sugars, sucrose, glucose, fructose, and glyco-

sidic-glucose in the pulp of the ripening fruit are shown in Fig. 8. As in the curves for the percentage amounts, Fig. 4, little change is observed for each sugar during the first twenty-four hours and sucrose obtained its peak value by the 7th day as the fruit became 'eating-ripe'. Sucrose then fell rapidly while
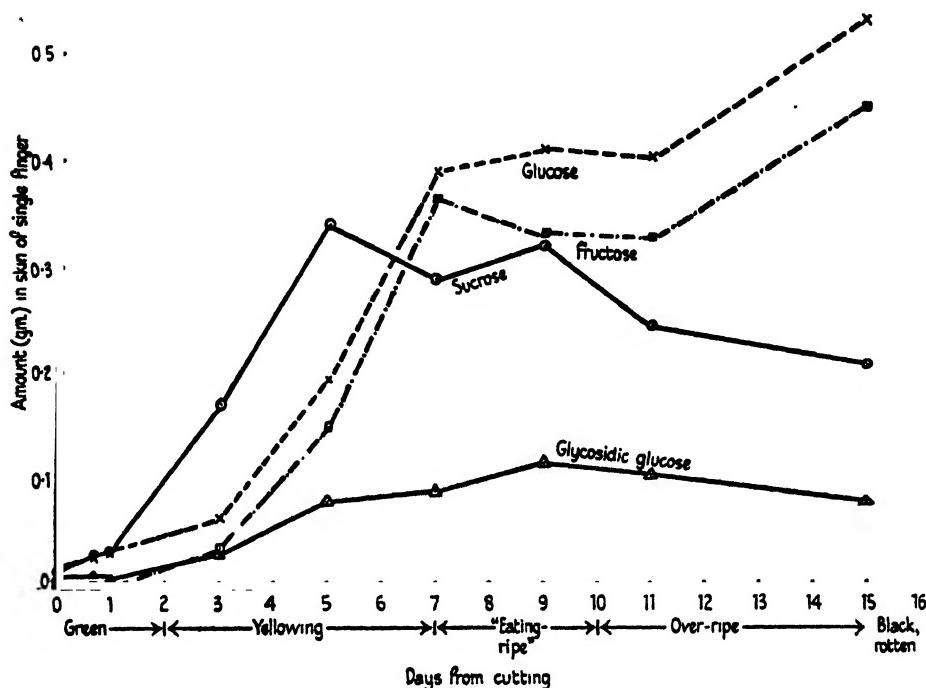
FIG. 9. Skin. Amounts (gm.) of sucrose, glucose, fructose, and of glycosidic-glucose in skin of single finger at the various stages of ripening of 'heavy ¾-full' fruit stored under tropical conditions.

the fruit was 'eating-ripe', reaching a relatively low value on the 11th day, which was maintained during the late senescent phase.

Glucose and fructose were present in approximately equal amounts in the green fruit though in less amount than sucrose. Glucose increased more rapidly than fructose or sucrose between the 3rd and 5th days and surpassed sucrose in amount by the 5th day. Both glucose and fructose attained their peak values on the 9th day, two days earlier than shown by their percentage amounts (Fig. 4).

For glycosidic-glucose (Fig. 8) the curve for total amount is similar in form to the curve of its percentage amount (Fig. 4).

(c) *Titratable acid.*

The expression of the titratable acid of the pulp as total amounts per pulp adds little to the information derived from the percentage results. The data

are given in column 4 of Table I. A peak value was observed during the 'eating-ripe' stage on the 9th day, but the observation for the 7th day was little below it. Increasing acid content is apparently associated with ripening, so this attainment of the high value in the pulp by the 7th day suggests that ripening is proceeding earlier in the pulp than in the skin, where the highest value was clearly not attained until the 9th day (column 5, Table I).

In general, the change of the basis of calculation of the data has had relatively little effect on the trends of the constituents of the pulp, a reflection of the maintained general level of the mean pulp weight throughout ripening (Fig. 1).

VIII. Changes in the Total Amounts per Finger of Dry Matter and Various Carbohydrates in the Skin

(a) *Total dry matter, starch, and total sugars.*

The effects of the pronounced trend in water content of the skin (Fig. 1) are well shown by a comparison of the dry matter and total sugar curves in Figs. 3 and 7. After the 5th day the percentages of dry matter and of total sugars increased due to the rapidly falling water content, while the amount of dry matter calculated per skin of a single finger fell and the total sugars remained approximately constant.

The drift of the total amount of dry matter in the skin per single finger, shown in Fig. 7, differed from that given by the pulp (Fig. 6) in its failure to show two phases of more rapid decrease. There was a relatively slight fall from the 1st to the 3rd day, becoming more pronounced between the 3rd and 5th days. This was followed by a greater rate of decrease from the 5th to the 11th day; after the 11th day to the 15th there was little change.

The total amount of sugars rose for the first sample, reaching a maximum value about the 7th day and maintaining this value into the over-ripe stage at the 11th day; between the 11th and 15th days a small increase occurred. The starch curve is little different from that shown for the percentage amounts in Fig. 3.

(b) *Sucrose, glucose, fructose, and glycosidic-glucose.*

Trends similar to those shown in the percentage curves, Fig. 5, were observed for each constituent up to the 5th day, but after that date they diverged widely as the effects of the changing water content became apparent.

Sucrose increased in amount until the 5th day but afterwards decreased. The method here adopted of presenting the data confirms that during the 'eating-ripe' and over-ripe stages there is an increase in the actual amounts of hexose sugars in the skins, especially in the latest stages of senescence. Glycosidic-glucose shows little difference in the form of its curve from that shown for percentage amounts (Fig. 5).
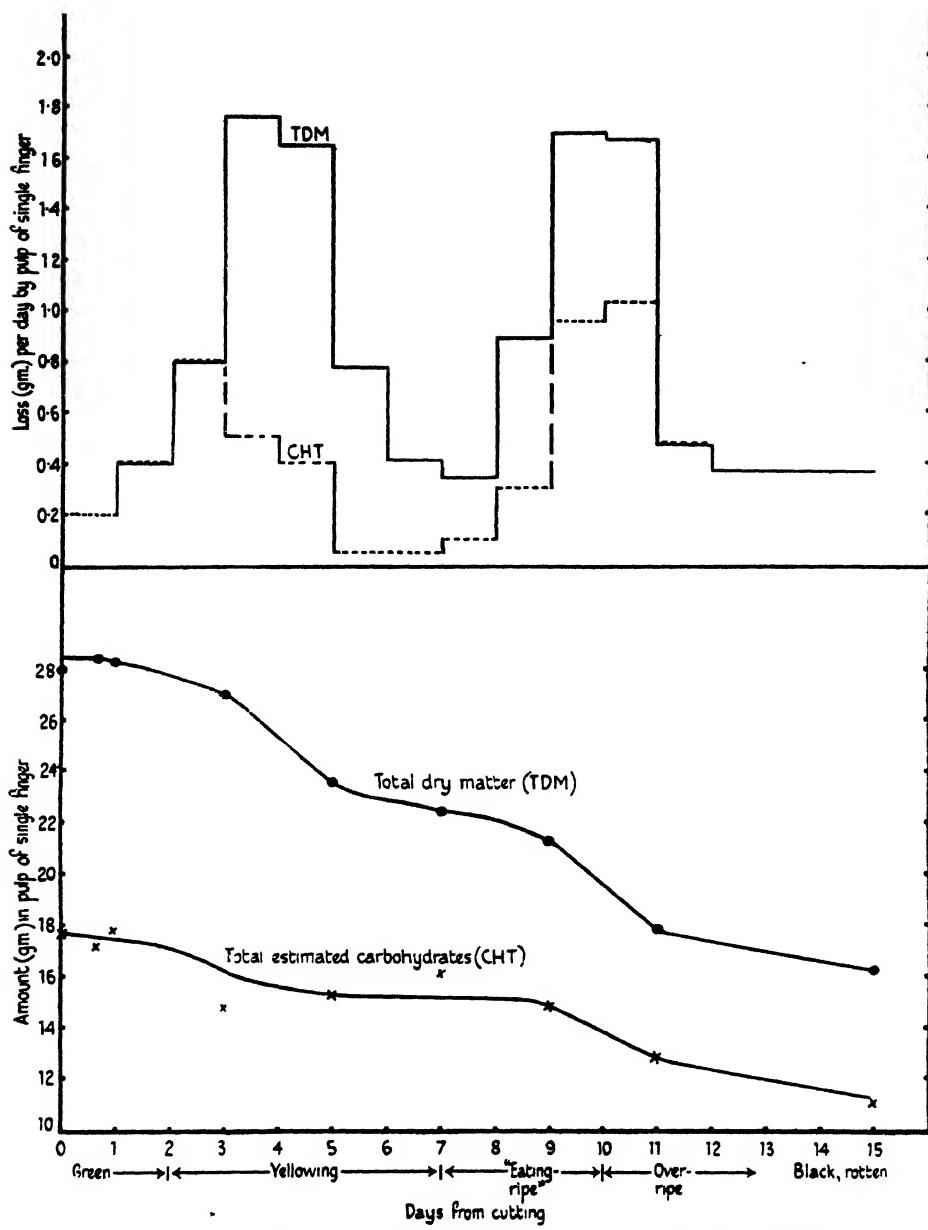
FIG. 10. Pulp. The smooth curve TDM in the lower portion of the figure has been drawn through the values given in Table V (Appendix) of the amounts (shown by dots) of total dry matter present in the pulp at each sampling. Similarly the curve CHT has been drawn through the amounts, denoted by crosses, of total estimated carbohydrates (starch + sucrose + glucose + fructose + glycosidic-glucose) in the pulp at each sampling.

In the upper portion of the figure the columns TDM give the daily rates of loss of total dry matter throughout the ripening period; the plotted values were obtained by calculation from amounts given by the curve TDM in the lower portion of the figure and are set out in column (2) of Table III. The columns CHT show similarly the daily rates of loss of total estimated carbohydrates through the same period; the plotted values were obtained by calculation from the amounts in curve CHT in the lower portion of the figure and are set out in column (3) of Table III.

(c) *Titratable acid.*

Values for the total amount of titratable acid in the skin during ripening are given in column 5 of Table I. The early stages of ripening were marked by falling values of acid in the skin, but a peak value was obtained on the 9th day during the 'eating-ripe' stage. This was two days earlier than the peak value given by the percentage data.

IX. Utilization in Respiration of Dry Matter and of Estimated Carbohydrates[1]

From the data of total dry matter and of estimated carbohydrates (starch, sucrose, glucose, fructose, and glycosidic-glucose) present in a single pulp or a single skin given in Tables V and VI of the Appendix the curves of Figs. 10, 11, and 12 have been constructed. In each figure the lower portion consists of values plotted for the dry matter and the total estimated carbohydrates, with smooth curves drawn through them. From these curves the values given in Table III for the daily loss of dry matter and the daily loss of the estimated carbohydrates have been obtained by inspection. These values have been plotted in the upper portions of Figs. 10 to 12 as stepped graphs.

Table III

Daily Losses of Total Dry Matter and of Total Estimated Carbohydrates in Pulp, Skin, and Whole Finger

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	Pulp.		Skin.		Whole finger.		
Day.	Total dry matter. (gm.)	Total estimated carbo-hydrates. (gm.)	Total dry matter. (gm.)	Total estimated carbo-hydrates. (gm.)	Total dry matter. (gm.)	CO_2 output. mg./kg./hr.	Total estimated carbo-hydrates. (gm.)
1	0·20	0·22	0·08	0·21	0·28	105	0·43
2	0·40	0·40	0·10	0·37	0·50	188	0·77
3	0·80	0·80	0·07	0·41	0·87	326	1·21
4	1·75	0·50	0·15	0·02	1·90	713	0·52
5	1·65	0·40	0·16	0·03	1·81	679	0·43
6	0·77	0·05	0·23	0·02	1·00	377	0·07
7	0·41	0·05	0·23	0·02	0·64	240	0·07
8	0·34	0·10	0·22	0·02	0·56	210	0·12
9	0·89	0·30	0·21	0·02	1·10	413	0·32
10	1·69	0·95	0·21	0·03	1·90	713	0·98
11	1·67	1·03	0·23	0·04	1·89	709	1·07
12	0·47	0·47	negligible	−0·03	0·47	176	0·44
13	0·36	0·36	,,	−0·05	0·36	135	0·31
14	0·36	0·36	,,	−0·06	0·36	135	0·30
15	0·36	0·36	,,	−0·05	0·36	135	0·31

[1] The discussion in this section, based as it is on comparatively few data, is to be regarded as a purely tentative approach to a wide and complex problem. It should also be observed here that the best supplies of fruit now available in Trinidad, through disease and other causes, do not permit sufficiently uniform ripening for rigid quantitative hypotheses to be based on the analytical data obtained from them.

Column 7 of Table III gives values for carbon dioxide production per hour per kg. based on the data of column 8, assuming dry matter loss was hexose only and that respiration was completely aerobic.

The rate of loss of dry matter gives a direct measure of the loss by respiration plus losses due to various volatile substances; the rate of loss of estimated carbohydrates indicates the proportion of this total loss for which these carbohydrates may have been responsible.

In the curve of dry matter loss of the pulp, TDM of Fig. 10, two peaks are observed. The rate rose over the first three days to a peak on the 4th day and after the 5th day quickly declined. After the 8th day the loss ·curve rose rapidly to another peak value at about the 10th day, as the fruit became overripe, and then fell during the last four days. The possible interpretation of these variations in rate of loss are discussed below.

The curve for the rate of loss of carbohydrates (curve CHT of Fig. 10, upper portion), admittedly based on less consistent data than the dry matter, shows considerable departures in magnitude and form from the curve of dry matter loss.

Since the rate of dry matter loss of the pulp may be considered as loss by respiration, and since the estimated loss of carbohydrate was at times considerably less than the dry matter loss, particularly at the peak values, it follows that sources of sugars for glycolysis other than those estimated were drawn upon in the pulp of the banana, particularly at the peak respiration rates. Fig. 11 presents graphically similar quantities for the skin.

A considerably larger and increasing rate of loss of estimated carbohydrates than of total dry matter occurred during the first three days (column 5 in Table III and curves CHT and TDM of Fig. 11, lower portion), leading to the conclusion that one or more of the estimated carbohydrates was undergoing transformation into some other substance appearing in the total dry matter, i.e. not migrating from the skin to the pulp. After the peak value on the 3rd day the rate of loss of estimated carbohydrates quickly declined to a very low value which was maintained until the 11th day, after which the quantity of estimated carbohydrates apparently increased; there was an apparent negative rate of loss, possibly due to sugar formation from the breakdown of hemicelluloses or other substances.

For whole fingers the total dry matter values and the total amounts of estimated carbohydrates per single pulp and per single skin, given in Tables V and VI of the appendix, have been added and the totals for each plotted in the lower portion of Fig. 12. From these curves the rates of loss of dry matter and of total estimated carbohydrates have been calculated and are presented in columns 6 and 8 of Table III and as curves TDM and CHT of Fig. 12, upper portion. The curves of the results for the whole fingers shown in Fig. 12 are similar in form to those for the pulp alone (Fig. 10), since this comprises the bulk of the dry matter and total estimated carbohydrates. For the whole finger there was at first a greater rate of loss of estimated carbo-

hydrates than of total dry matter, but after three days the rate of loss of esti-
mated carbohydrates fell, as in the pulp, to values which were very small
relative to the dry matter rate. The rate of dry matter loss for the whole finger
presented a curve showing two peaks (3–5 and 9–11 days) as in the pulp, but
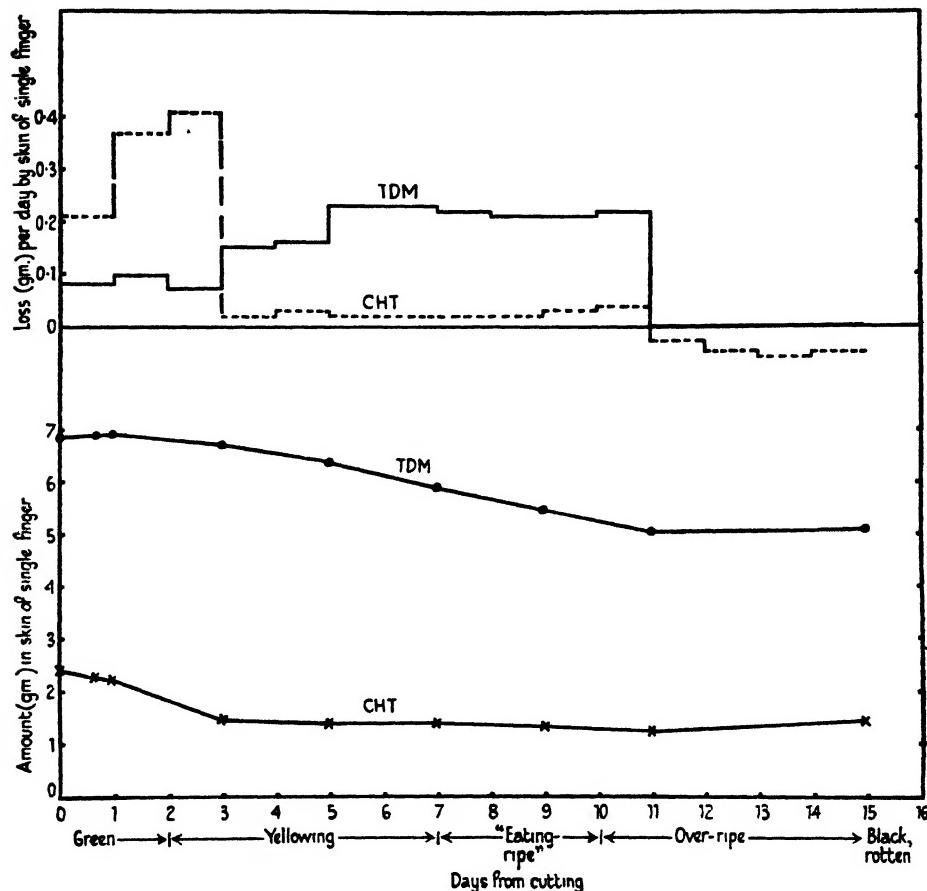
Fig. 11. Skin. Calculations for the skin similar to those for the pulp shown in Fig. 10.
Corresponding data are given in Table VI (Appendix) and columns (4) and (5) of Table III.

the peaks were somewhat higher than for the pulp alone, and the intervening
values (5–9 days) were also higher. This drift, shown by curve TDM in Fig. 12
(upper portion), of the rate of loss of total dry matter should in the main repre-
sent loss due to respiration of the entire fruit. By regarding net loss of dry
matter as loss resulting from the aerobic breakdown of hexoses in respiration[1]

[1] In fact the respiration process of the banana during ripening passes through certain unde-
fined phases, including aerobic (both full and partial oxidation of carbohydrates), mixed
aerobic, and anaerobic; in late senescence it is probably mainly anaerobic. When anaerobic
respiration is proceeding to any appreciable extent, estimates of the respiration rate based on
rates of loss of dry matter will be considerably higher than those based on measurements of
carbon dioxide liberation.
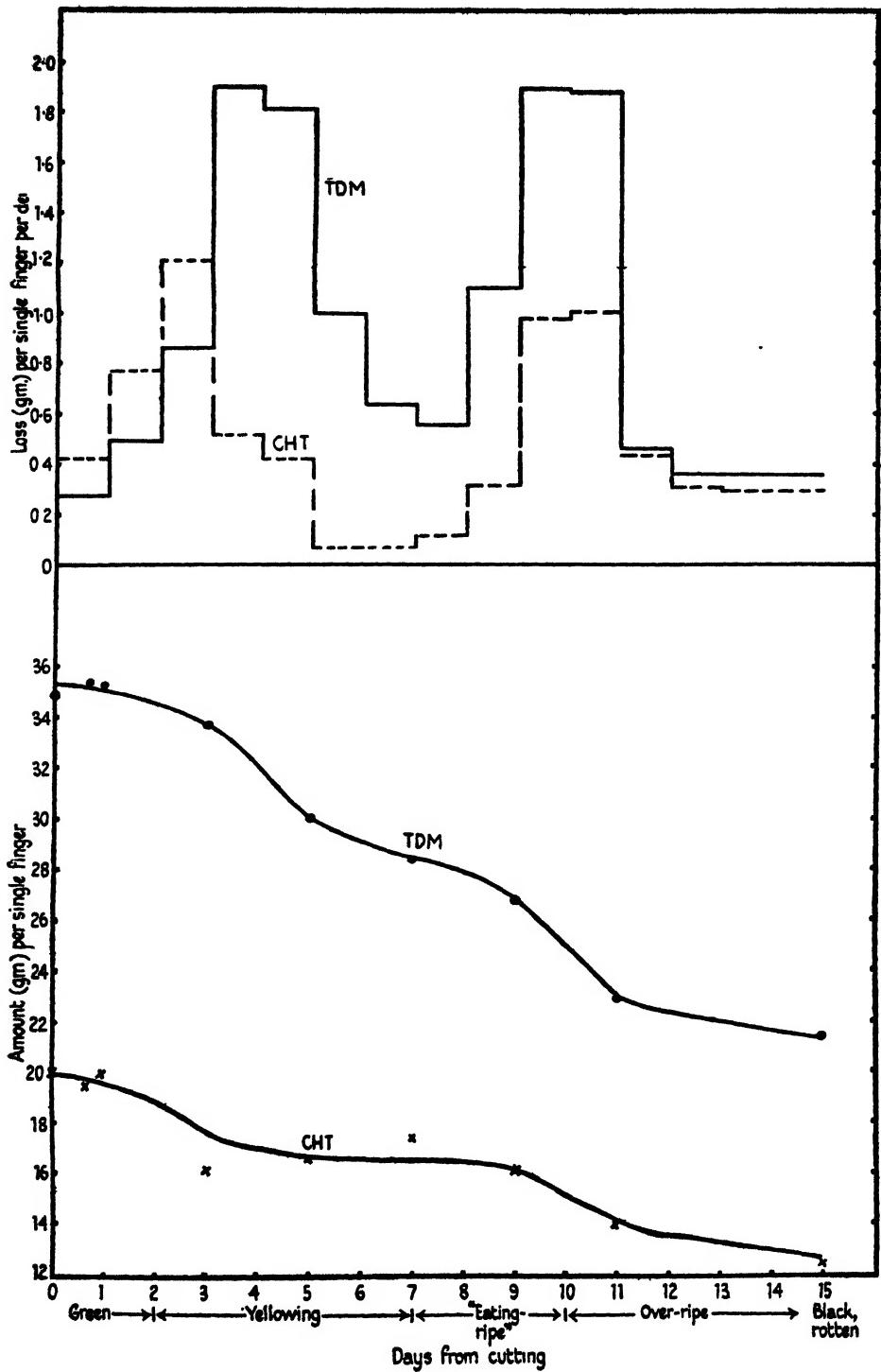
FIG. 12 (see opposite).

the rates of carbon dioxide production have been calculated as mg. per hour per kg. of fruit and are given in column 7 of Table III. These calculated respiration rates show that the banana (as a statistical unit), ripening while attached to the bunch at fluctuating diurnal tropical temperatures, attains a peak respiration rate associated, after about three days, with the onset of yellowing of the skin and then, after an interval of relatively low respiration activity, attains, after nine days, another peak rate of approximately the same intensity, during and towards the end of the 'eating-ripe' stage. A similar sequence of rises and falls in the respiration rate of the banana has been found by Wardlaw and Leonard (1940) by direct measurement of carbon dioxide liberation in fruit ripened at 85° F. at 100 per cent. relative humidity, but the amounts of carbon dioxide liberated were, at each stage, considerably lower than those calculated here.

TABLE IV

Changes in Residue during Ripening [1]

Time from cutting.	Pulp.		Skin.	
	Alcohol-insoluble Residue.	Alcohol-soluble Residue.	Alcohol-insoluble Residue.	Alcohol-soluble Residue.
o	9·32	1·00	3·91	0·54
16 hrs.	9·61	1·66	3·96	0·65
23 ,,	9·23	1·23	4·14	0·58
3 days	11·54	0·73	4·40	0·91
5 ,,	5·77	2·56	4·00	1·07
7 ,,	4·16	2·22	3·68	0·88
9 ,,	3·26	3·13	3·57	0·66
11 ,,	2·51	2·50	3·30	0·55
15 ,,	2·64	2·62	3·19	0·51

[1] The alcohol-insoluble residue and the alcohol-soluble residue fractions are respectively starch-free and sugar-free.

In Table IV and curves A and B of Fig. 13 the values are given for the alcohol-insoluble residue per pulp and skin of single fingers. This residue is shown to be a source of respirable material in addition to that of the estimated carbohydrates. In the pulp (curve A of Fig. 13) there was a considerable fall in the total amount of this fraction from the 3rd day until the 11th day, the fall gradually decreasing in rate and then remaining constant. A portion of

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FIG. 12. Whole Finger. The combined amounts for pulp and skin of total dry matter (TDM) and of total estimated carbohydrates (CHT), respectively, have been plotted in the lower portion of the figure and smooth curves drawn through the points.

In the upper portion of the figure graph TDM gives the daily rates of loss of total dry matter by the whole finger throughout the ripening period, the plotted values were obtained by calculation from the amounts in the curve TDM in the lower portion of the figure and are set out in column (6) of Table III. Graph CHT shows the daily rates of loss of total estimated carbohydrates through the same period; the plotted values were obtained by calculation from the amounts in the curve CHT in the lower portion of the figure and are set out in column (8) of Table III.

this loss of alcohol-insoluble material is to be explained by the increase in alcohol-soluble residue (A.S.R. in curve A of Fig. 13) during the same period, i.e. there is a transformation of alcohol-insoluble material to alcohol-soluble,
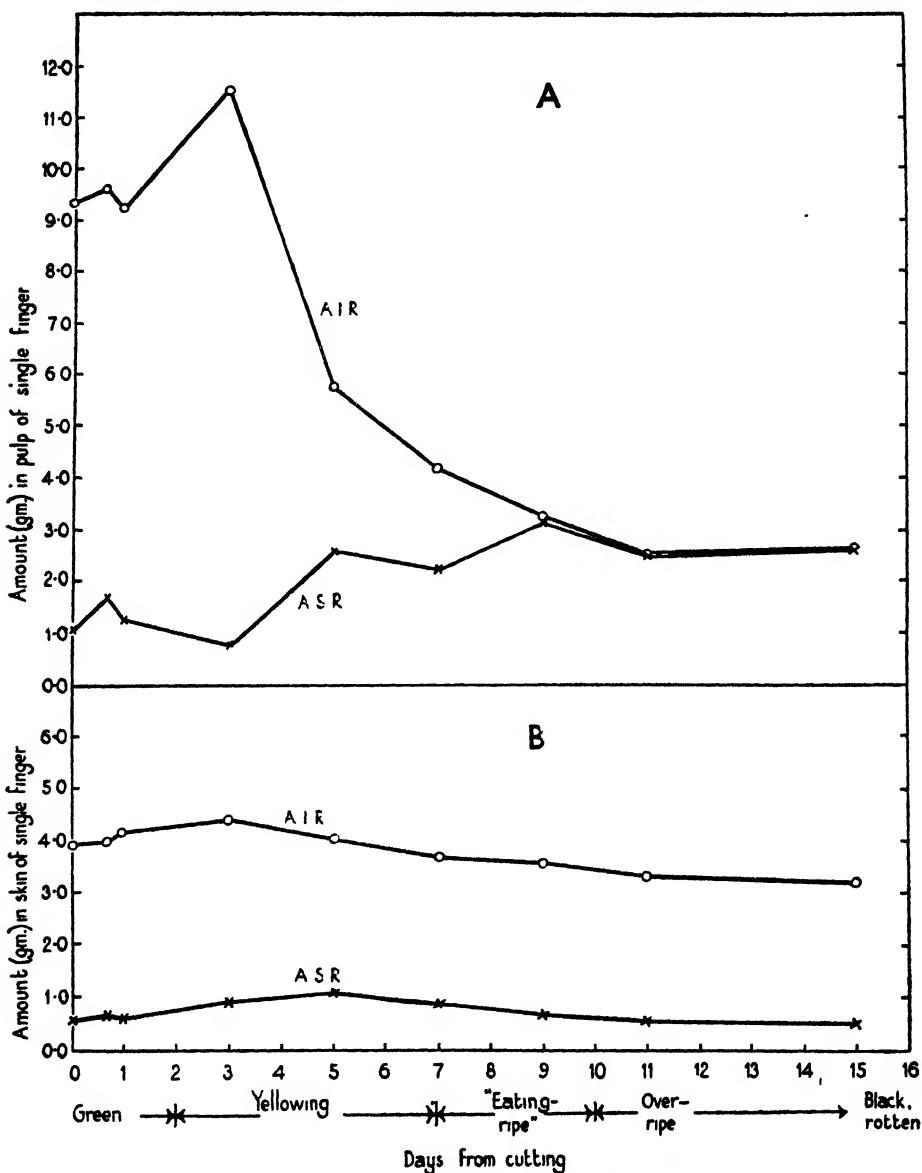
FIG. 13. A: Pulp; B: Skin. Amounts of alcohol-insoluble residue, less starch (A.I.R.), and alcohol-soluble residue (A.S.R.) at the various stages of ripening.

non-estimated material. However, this evidence in conjunction with that of Fig. 10 shows that at the climacteric, and shortly after, the major supply of respirable material in the pulp is to be found in the non-starch fraction of the

alcohol-insoluble substance; this material may be of the nature of labile cell-wall substance.

In the skin, except between the 3rd and 5th days, the fall in alcohol-insoluble residue was not accounted for, to any extent, by increases in the alcohol-soluble fraction (curve B of Fig. 13). The change in both curves was less striking than for the pulp, but it again suggests that the alcohol-insoluble residue provides material for respiration but that in the skin the conversion to alcohol-insoluble residue after the 5th day does not proceed at a rate sufficient to maintain it at a constant level.

X. DISCUSSION

The consideration of the carbohydrate metabolism of the banana fruit when attached to the plant, given in a previous communication (Barnell, 1940), has now been extended to a study of the changes which occur in the fruit when the bunches are cut from the plant at a particular developmental stage and maintained at fluctuating, tropical temperatures. Fruit, which if left on the plant for a further 10–20 days would have remained green and continued to increase in size with little change in composition, showed on cutting definite ripening changes within the first twenty-four hours. During this period weight was lost by the whole finger, each of the sugars increased in concentration in both pulp and skin, and the starch content began to fall. By the 3rd day, when colouring was just observable in two bunches only out of thirty, the chemical changes had proceeded to a considerable extent; the starch content had fallen by approximately 20 per cent. in the pulp and over 50 per cent. in the skin, while the sugars, particularly sucrose, increased in both components by several hundred per cent. These ripening changes continued until by the 7th day the starch in skin and pulp had practically disappeared, while the sugars continued to increase in concentration due, in the later stages, to loss of water by transpiration from skin and pulp. During the period of rapidly increasing sugar concentration in the pulp from the 3rd to the 9th day the pulp gained in fresh weight though the whole finger lost weight. The suction-pressure of the pulp cells, due to their high concentration of osmotically active solutes, was sufficient to cause the cells of the pulp to withdraw water from the skin. After the 9th day changes occurred in the cell organization which rendered this high suction-pressure incapable of withdrawing water from the skin and water was rapidly lost from all tissues.

All three sugars, sucrose, glucose, and fructose, increased as ripening advanced; in both pulp and skin, sucrose was at first the dominant sugar. In the pulp, sucrose attained a maximal value just as the fruit became 'eating-ripe' and then fell, while the reducing sugars had already exceeded the sucrose concentration at the beginning of the 'eating-ripe' stage and continued to increase during that stage. In the skin the percentage amount of sucrose continued to increase throughout the 'eating-ripe' stage; this sugar was, however, exceeded in concentration during this stage by both reducing sugars,

particularly by glucose. 'Bound' or glycosidic-glucose was never present in large amount; in the pulp it increased to the 5th day and then decreased throughout the ripening and post-ripe stages, while in the skin it continued to increase during the ripe stage but fell during late senescence.

Changes in the titratable acid of the pulp and skin were associated with ripening, rising values accompanying yellowing but with a time delay in the skin of about two days compared with the pulp. It is suggested that acidity changes may be connected with changes in the mechanism of the respiratory process, and that correlated studies of respiration and acid metabolism are required.

The presentation of the data as total amounts of dry matter and of each estimated substance present in the pulp and skin of a single finger enables the changes in amounts of each substance to be observed without the distortion produced by such a variable basis as the fresh weight. From the curves of total dry matter and total estimated carbohydrates the rates of loss of dry matter and of the carbohydrates were plotted. The rates of loss of dry matter may be taken as approximations to the respiration rates of the tissues; the rates of loss of estimated carbohydrates should give some indication of the part played by these metabolites in providing the carbohydrate substrate of respiration, assuming that transformation into other substances does not take place. The conditions of ripening—fluctuating temperatures and humidity and the fact that fruit remained attached to the bunch until sampling in these studies—may be responsible to some extent for the considerable differences observed between this method of assessment of respiration rate and that by direct measurement of carbon dioxide liberated from individual fingers in a constant environment (Wardlaw and Leonard, 1940), but the occurrence of anaerobic respiration in varying proportions will also produce large differences.

In the pulp the rates of dry matter loss showed two well-defined peaks, one coinciding with the time the fruit reached the 'sprung' condition and the other with the attainment of the ripe to over-ripe stage.

The rate of loss of estimated carbohydrates was at times much lower than the rate of loss of total dry matter, so it follows that sources other than these carbohydrates were drawn upon in the respiratory metabolism of the pulp. The alcohol-insoluble residue has been shown to be an additional major source of respirable material.

In the skin the rate of loss of estimated carbohydrates (Fig. 11) was considerably greater during the first three days than the rate of loss of total dry matter, and it must be considered that during this period some of these carbohydrates were undergoing transformation into other compounds.

The summation of the data for the skin and the pulp gives the rates of loss of dry matter and of estimated carbohydrates for the whole finger (Fig. 12) as a preliminary step in the comparison with direct estimation of carbon dioxide liberation rates and of internal gas concentrations now being carried out in this department.

The considerable changes in composition which, within twenty-four hours of cutting, have been detected in the pulp and skin of fruit held at tropical temperatures direct attention to the importance, for any prolonged storage, of quick cooling of the fruit. Investigations now in progress on the biochemistry of fruit cut at the '¾-full' and 'heavy ¾-full' grades and subjected to various cold storage treatments will permit the comparison, in a future communication, of the compositions of such fruits at the 'eating-ripe' stage with that of fruit ripened at tropical temperatures.

XI. Summary

1. In the Gros Michel banana various changes have been followed during the ripening of detached bunches of a commercial grade held at tropical temperatures.

2. The fresh weight of the whole finger fell throughout the ripening period, but most rapidly between 2–3 days and 9–11 days after cutting the bunch. The pulp lost fresh weight till the 3rd day, then increased in weight till the 9th day, after which a loss again occurred. The skin lost weight all through the period, but particularly quickly between the 9th and 11th days.

3. The percentage of dry matter in the pulp decreased as the fruit ripened. Starch had fallen to small values at the 'eating-ripe' stage, while the total sugars rose to a peak value at the beginning of this stage. Sucrose attained its peak value at the beginning of the 'eating-ripe' stage and then fell, while reducing sugars continued to increase in percentage amount till the fruit became over-ripe. Glycosidic-glucose, which was present in small amount only, increased during the 'eating-ripe' and over-ripe stages. Titratable acid decreased in the pulp till the 'sprung' condition was reached and then increased as colouring occurred, falling again during late senescence.

4. In the skin the percentage amount of dry matter increased all through the period of observation, but particularly during the 'eating-ripe' and over-ripe stages. Increase during the 'eating-ripe' stage is ascribed particularly to loss of water to the pulp and in the over-ripe stage to high transpiration losses. The starch content of the skin fell to negligible values at the 'eating-ripe' stage, while total sugars increased slightly during the 1st day and then steadily to the end of the period. Sucrose increased in concentration till the fruit was rotten. Glucose was in excess of fructose and both sugars increased till the end of the period. The percentage amount of each sugar throughout was much lower than in the pulp. Glycosidic-glucose increased consistently till the fruit was over-ripe, falling slightly during this last stage. It was never present in large amount. Titratable acid increased in the skin as ripening proceeded, though with a time-lag compared with the pulp.

5. In both the pulp and skin of the green banana the alcohol-soluble material contained a high proportion of non-sugars. The proportion decreased as sugars increased in concentration during ripening.

6. The data have also been presented as amounts in the pulp and in the skin of a single finger so that the changes in amount of each constituent during ripening may be followed without the distortion produced by reference to the changing basis of fresh weight.

7. From the drifts of the total amount of dry matter in pulp and in skin the rates of loss of dry matter per day have been calculated. Since these rates represent in the main the loss of matter from the tissues by respiration it becomes possible to follow the drift of the respiration process in the pulp and the skin separately and also in the whole finger while the finger remains attached to the bunch. The rates of loss of dry matter of the pulp show two peak values, one as the fruit enters the sprung condition, and the other as the fruit passes from 'eating-ripe' to over-ripe. The skin showed a rising rate of dry matter loss over the first five days, then a steady value till the 11th day, after which a decline occurred. Results for the whole fruit are presented for subsequent comparison with measurements of carbon dioxide liberation and internal gas concentrations.

8. A comparison of rates of loss of total estimated carbohydrates (starch, sucrose, fructose, and glycosidic-glucose) with those of the total dry matter showed that in the pulp the carbon substrate for respiration was at some stages derived, at least in part, from sources other than the estimated carbohydrates; while in the skin the rate of loss of estimated carbohydrates at times exceeded the rate of loss of dry matter, thus indicating the transformation of some carbohydrate into a substance or substances which were not estimated.

9. The non-starch fraction of the alcohol-insoluble substance has been shown to be an important source of respirable material in both pulp and skin.

10. The need for quick cooling of bananas after cutting is clearly demonstrated by the rapid chemical changes observed in the fruit during the first few hours under tropical conditions.

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APPENDIX. TABLE V

Pulp Weight and Amounts (gm.) of Various Constituents in Pulp of Single Finger

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Time from cutting.	Pulp weight. (gm.)	Dry matter.	Glucose.	Fructose.	Sucrose.	Total sugars.	Starch.	Glyco-sidic-glucose.	Total estimated carbo-hydrate.
0	100·3	28·00	0·024	0·024	0·049	0·097	17·50	0·018	17·62
16 hrs.	100·5	28·41	0·026	0·030	0·069	0·125	16·99	0·020	17·14
23 ,,	99·5	28·21	0·043	0·030	0·108	0·181	17·56	0·012	17·75
3 days	97·0	27·00	0·270	0·238	0·767	1·275	13·41	0·040	14·73
5 ,,	100·2	23·60	3·68	2·98	3·59	10·25	4·61	0·411	15·27
7 ,,	104·7	22·42	5·09	5·13	3·83	14·05	1·63	0·360	16·04
9 ,,	108·4	21·19	6·25	5·75	1·84	13·84	0·709	0·246	14·80
11 ,,	97·3	17·83	5·89	5·42	0·963	12·27	0·348	0·206	12·82
15 ,,	98·9	16·28	4·82	4·84	0·989	10·65	0·300	0·068	11·02

TABLE VI

Skin Weight and Amounts (gm.) of Various Constituents in Skin of Single Finger

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Time from cutting.	Skin weight. (gm.)	Dry matter.	Glucose.	Fructose.	Sucrose.	Total sugars.	Starch.	Glyco-sidic-glucose.	Total estimated carbo-hydrate.
0	62·81	6·86	0·019	0·002	0·015	0·036	2·375	0·009	2·41
16 hrs.	62·29	6·90	0·027	0·002	0·029	0·058	2·224	0·012	2·29
23 ,,	62·04	6·94	0·033	0·002	0·033	0·068	2·144	0·007	2·22
3 days	59·85	6·73	0·064	0·036	0·171	0·271	1·115	0·030	1·42
5 ,,	56·20	6·42	0·194	0·150	0·341	0·685	0·576	0·080	1·35
7 ,,	49·64	5·94	0·390	0·365	0·290	1·045	0·238	0·090	1·38
9 ,,	42·36	5·53	0·412	0·333	0·322	1·067	0·113	0·116	1·30
11 ,,	28·98	5·06	0·404	0·329	0·246	0·979	0·123	0·106	1·21
15 ,,	26·00	5·11	0·534	0·452	0·209	1·195	0·134	0·081	1·41

The Diagrammatic Representation of the Results of Physiological and other Experiments Designed Factorially

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With four Figures in the Text

THE rational methods of experimental design initiated by Fisher and his school (Fisher, 1937; Yates, 1937) have aroused wide interest in recent years. Experiments designed on factorial principles are highly efficient, in that they yield the maximum of information; and they aim from the outset to provide data in a form suitable for a full statistical analysis. By reason of the merits of high efficiency and abundance of information the results of such experiments are not always easy to present clearly, fully, and concisely in diagrammatic form. Although ingenious diagrammatic representations have been proposed from time to time, nevertheless it may be useful to describe a new type of diagram which, for simpler factorial experiments, has been found to possess to a high degree the desirable qualities enumerated above. The more complex factorial diagrams, if aiming at completeness, inevitably lose in clarity, so that here the alternatives become either a complete and concise but complex diagram or a series of simpler diagrams in which some available information is sacrificed.

The type of diagram under consideration may most easily be described by means of an example. An experimental layout frequently employed consists of combinations of three factors each operating at two levels; and in one such experiment on mineral nutrition carried out at this Institute the variable factors concerned were phosphorus, potassium, and rubidium supply. Barley was grown in a high calcium solution at two levels of each of the first two elements, both in the absence and presence of the third. Data of the water content of the stem will be considered. If the *higher levels* of the variables are designated P, K, and R respectively, the eight resultant treatments may be denoted by O, P, K, R, PK, PR, KR, and PKR. The mean water contents, together with the calculated magnitudes of the various effects corresponding to the seven individual degrees of freedom (cf. Yates, 1937, p. 15) are given in Table I. Each water content is the mean of nine observed values.

From the point of view of experimental design the eight treatments fall naturally into four groups: (1) O, with all factors at the lower level; (2) P, K, and R, with two factors at the lower level and one at the higher; (3) PK, PR,

TABLE I

Water Content (% dry wt.) and Effect

Treatment.	Water content.	Mean effect.
O	669	—
P	791	+70·0*
K	655	+17·0
PK	682	−47·5*
R	453	−142·0*
PR	566	−4·5
KR	596	+78·5*
PKR	614	0·0
Standard error	±17·4	±12·31

Significance levels of mean effects: 5% : 24·8; 1% : 33·1.
* Significant result at 1% level.

and KR, with two factors at the higher level and one at the lower; and (4) PKR, with all factors at the higher level. In the diagram (Fig. 1) these four groups are inserted in the order named at equal intervals along the abscissa, and along the appropriate ordinates are plotted the observed water contents in the usual way, giving eight treatment points. These eight points are interconnected by a series of twelve lines, the difference in height of the extremities of each line (i.e. the slope) representing the difference in water content resulting from the difference in level of a selected element. The lines in the diagram therefore constitute three sets of four, and on the diagram these are ··drawn differently, black representing a difference of phosphorus level, white of potassium level, and pied of rubidium application. The diagram may thus be traversed from treatment O to PKR along the various lines by six different routes, on each of which it is necessary to follow one line and one only of each type.

In the diagram so constructed there may be distinguished six quadrilaterals, which may be subdivided into three groups of two. One pair, bounded by black and white lines, represents the interactions of phosphorus and potassium in the absence and presence of rubidium (i.e. O, P, K, PK, and R, PR, KR, PKR respectively); the other two pairs similarly represent interactions of phosphorus and rubidium at both levels of potassium, and of potassium and rubidium at both levels of phosphorus. Owing to the manner of grouping and spacing of the treatment points, the interaction represented by any one quadrilateral approaches zero as the figure approaches a parallelogram. The complete diagram thus represents conveniently the interactions of each pair of factors at both levels of the third, the nature and extent of the interactions being readily apparent to the eye.

Moreover, it is easy to insert on the diagram sets of four points to represent each of the three first-order interactions, or the whole second-order, the measure of the degree of interaction being given as before by the departure of these four points from a parallelogram. Thus the mid-point of each black line represents the mean value of two treatments differing only in phosphorus

level; the four points *o*, *k*, *r*, and *kr*, therefore represent the total first-order interaction between potassium and rubidium (K×R). From the mode of construction of the diagram these mid-points are necessarily equally spaced along the abscissa, two of the points lying on the central ordinate, and again
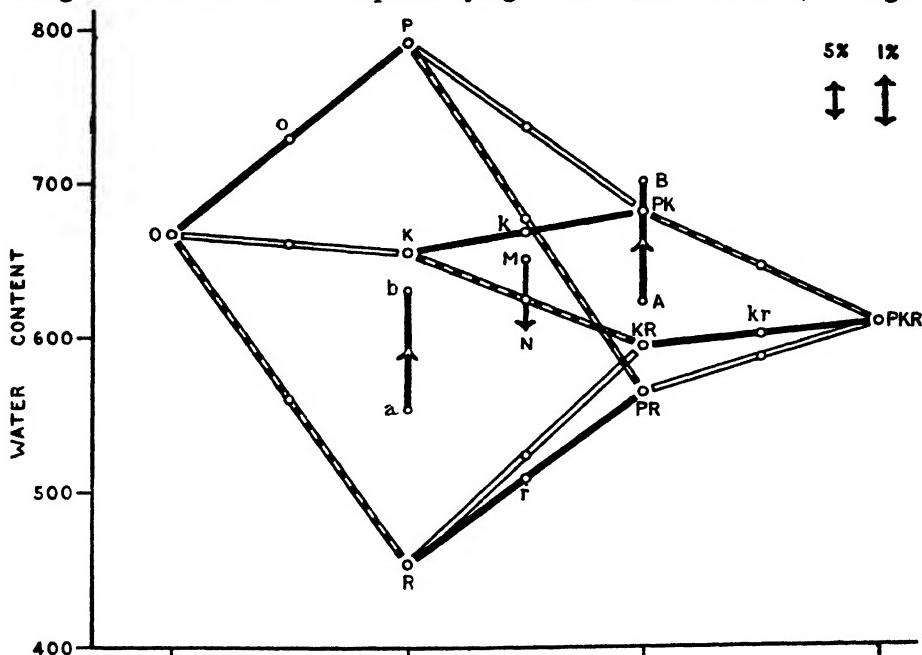
FIG. 1. Interaction diagram illustrating a 2×2×2 nutritional experiment on the water content (per cent. dry weight) of stems of barley; the variables are phosphorus level (P), potassium level (K), and rubidium application (R). For full explanation see text.

absence of interaction obtains when they define a parallelogram. The four points may be readily brought to the attention by placing a finger-tip close to each (using two fingers from each hand), when the kind and amount of inter-action is readily assessed. Similarly, the mid-points of white (K) lines repre-sent the interaction of phosphorus and rubidium (P×R), and those of the pied (R) lines the P×K interaction. In diagrams of this type, therefore, the mid-points of all constructional lines may usefully be inserted to enable a rapid survey of the various first-order interactions to be made.

If desired, the magnitude of any of these first-order effects may be shown directly as a line on the central ordinate of the figure. Thus the magnitude of the P×K interaction is given by the expression

$$\tfrac{1}{4}[(PKR+PK)+(R+O)]-\tfrac{1}{4}[(PR+P)+(KR+K)].$$

The first two treatments are situated at the extremities of the right-hand pied line, and the second pair at the extremities of the left-hand pied line. If, therefore, the distance between the mid-points of these lines is bisected in

N, the height of N measures the first half of the above expression. Similarly M, the bisector of the distance between the mid-points of the two central pied lines, measures the second half of the expression, and the magnitude of the interaction is given immediately by the length of the line MN. These two points are simply the bisectors of the diagonals of the quadrilateral representing the $P \times K$ interaction. If M falls below N the algebraic sign of the interaction is positive; if above, the sign is negative. It is useful to represent the sign by considering MN as a vector, hence an arrow-head has been inserted, pointing from M to N; the convention adopted is that a positive interaction is shown as an upward direction, and a negative one as a downward.

In order to represent the magnitude of the second-order interaction any one of three sets of four points may be inserted. Consider one of the three pairs of quadrilaterals into which the diagram may be resolved, e.g. the $K \times R$ interactions in the absence and in the presence of phosphorus. In its absence the quadrilateral is defined by the points representing treatments O, K, R, and KR, and bisection of the diagonals determines the vector ab, just as MN was obtained; ab thus gives the magnitude of the $K \times R$ interaction at the low phosphorus level and is algebraically equal to $\frac{1}{2}[(KR+O)-(K+R)]$. Similarly, from the quadrilateral at the high phosphorus level is obtained AB, equal to $\frac{1}{2}[(PKR+P)-(PK+PR)]$. From the construction a and b lie on the same ordinate, as also do A and B; ab and AB are necessarily parallel. If also aA is parallel to bB, then the summation of values corresponding to the two groups of treatments following are equal, viz.

$$(PKR+P)+(K+R) = (PK+PR)+(KR+O),$$

and there is no second-order interaction. Hence once again the measure of the total second-order interaction is the degree of departure of the points a A b B from a parallelogram. More precisely it is half the difference in magnitude between AB and ab, these being considered as vectors and not simply as lengths; if the direction from a to b is the same as that from A to B the difference between the two lengths gives twice the required quantity, whereas if the directions are opposite the sum of the lengths must be taken. The magnitude and sign may again of course be obtained geometrically by bisection of the diagonals aB and Ab, in just the same way as ab and AB were themselves obtained; though this final reduction is not usually necessary.

By considering either of the two other pairs of interactions ($P \times R$ at both levels of K and $P \times K$ at both levels of R) two other distinct sets of four points will be obtained, but if one of the three possible sets forms a parallelogram the others must both do likewise; while if one set departs from a parallelogram in the sense that the distance AB does not equal ab, then the other two sets will each have the same departure, this providing the measure of the interaction under consideration. The difference between the three sets of four points is readily understood by considering the magnitudes represented by the mid-points of ab and AB, points which may be usefully indicated by the tips of

the directional arrow-heads. These are respectively $\frac{1}{4}$(O+K+R+KR) and $\frac{1}{4}$(P+PK+PR+PKR). Since the treatments in the two bracketed expressions differ always and only in phosphorus dosage, the difference in height of the mid-points is the measure of the average or main phosphorus effect. Similarly, from the other two quadrilaterals also representing the second-order interaction may be readily deduced the magnitudes of the main potassium and rubidium effects, but since the consistency and magnitudes of these effects at all levels of the other factors may be immediately judged from the slopes of the constructional lines it is not necessary to insert the further complicating points into the diagram.

To render the diagram more complete statistically, lines whose lengths represent the 5 per cent. and 1 per cent. levels of significance of the seven individual effects have been inserted; an effect which may be reduced to a shorter line than these must be regarded as unproven. Of the three main effects that of phosphorus is the only one which is fully depicted and it is seen to be positive and highly significant. The lines drawn, however, are sufficient to show at a glance that the rubidium effect is negative, greater, and therefore even more significant than the phosphorus. The average potassium effect is obviously small and probably not significant; potassium may clearly have a real effect, but this depends for its direction as well as its magnitude on the levels of other factors. Both its first-order interactions are clearly real, that with phosphorus being reduced to the vector MN, which indicates immediately a negative and real effect. The total $K \times R$ interaction has not been reduced to a single vector, although the individual interactions at the high and low phosphorus levels are reduced to *ab* and AB respectively; the total interaction will be the mean of these two and is even larger than the $P \times K$ interaction, with the opposite sign. The third first-order interaction, $P \times R$, has again not been reduced, but is clearly of a very low order, considered as a whole or at either potassium level. Finally, the second-order effect is seen from a comparison of *ab* with AB to be completely negligible.

The use of the diagram is not necessarily confined to the visual separation of the effects as usually assessed, and indeed it may display prominently other types of relationships which, though intrinsically simple, do not lend themselves readily to statistical estimation along the more orthodox lines. This may occur when an effect which *in toto* is a single logical entity becomes subdivided by the analysis and distributed over several degrees of freedom. In the present instance the general effects of phosphorus and rubidium on water content are obvious in any examination of the data, however cursory; the simplicity of the highly important potassium effect may more easily be overlooked, and the arithmetical separation of the individual interaction effects does not immediately reveal it. On the other hand, examination of the general form of the structural lines in Fig. 1 shows immediately that the large and opposite effects of rubidium and phosphorus found at the low potassium level are very much reduced at the higher level. The physiological effect of

potassium is thus to reduce the effects of the other elements, and to stabilize or buffer the plant structures in relation to water content, a fact whose importance and probable cause the author has had occasion to point out elsewhere (Richards and Shih, 1940). The only two large and significant statistical effects of potassium in the experiment, namely the interactions $P \times K$ and $K \times R$, are in fact particular aspects of a general effect of the element, and the formal statistical subdivision of the effect scarcely helps to reveal the real nature and simplicity of the physiological action.

The main objection to be raised against the above method of representation is that frequently it will happen that points on the same ordinate will nearly coincide, leading to overlapping of lines. In certain cases this may give rise to undesirable confusion. Such a situation may be met by choosing different units of length along the abscissa to represent the three main factors. Thus as a modification of Fig. 1, application of P may be represented by 7 abscissa units, K by 5, and R by 10; PK will then be situated at 12 units along the horizontal axis, PR at 17, and KR at 15; finally PKR will be placed at 22 units. In other respects the diagram is completed exactly as is Fig. 1, and the interpretation of the two figures is exactly similar, since a parallelogram in one reappears as a parallelogram in the other. By these means not only can treatment points which coincide in the normal figure be separated to any desired extent, but also the lines *ab* and AB may be removed entirely from ordinates occupied by treatment points, thus eliminating all sources of confusion. Apart from a certain loss of symmetry, there is a real objection to such a modification in that the slopes of the three types of constructional lines are now no longer directly comparable and need to be interpreted in relation to their different horizontal scales; but in some instances the gain in clarity may more than offset this disadvantage.

The diagram from the $2 \times 2 \times 2$ experiment described above is readily adaptable to more complicated experiments of the 2^n type, though the complexity of the complete diagram increases rapidly with increasing n. Thus in the four-factor experiment the diagram will have sixteen treatment points interconnected by a network of thirty-two lines in four sets of eight. A hypothetical skeleton of this type is given in Fig. 2; it is designed to be free from statistical interactions of any order, and the method of construction and general interpretation should present no difficulty after becoming familiar with Fig. 1. The lines of Fig. 2 are identical with those of a projection in two dimensions of a four-dimensional figure, just as the lines of a diagram similar to Fig. 1, but without interactions, may be regarded as a two-dimensional projection of a three-dimensional parallelepiped. These diagrams thus present in one plane all the relations that would be exemplified in multi-dimensional models; but it is undesirable to attempt to visualize them as solids or models of higher order.

Fig. 2 is admittedly complex, but fortunately for nearly all purposes simpler diagrams will suffice. The system comprises six first-order interactions, four

second-order, and one third-order. Individual representation of the second-order effects would require four diagrams of the same order of complexity as Fig. 1, entailing loss of information about the third-order interaction only; and rarely is an interaction between four factors of any practical interest. Any
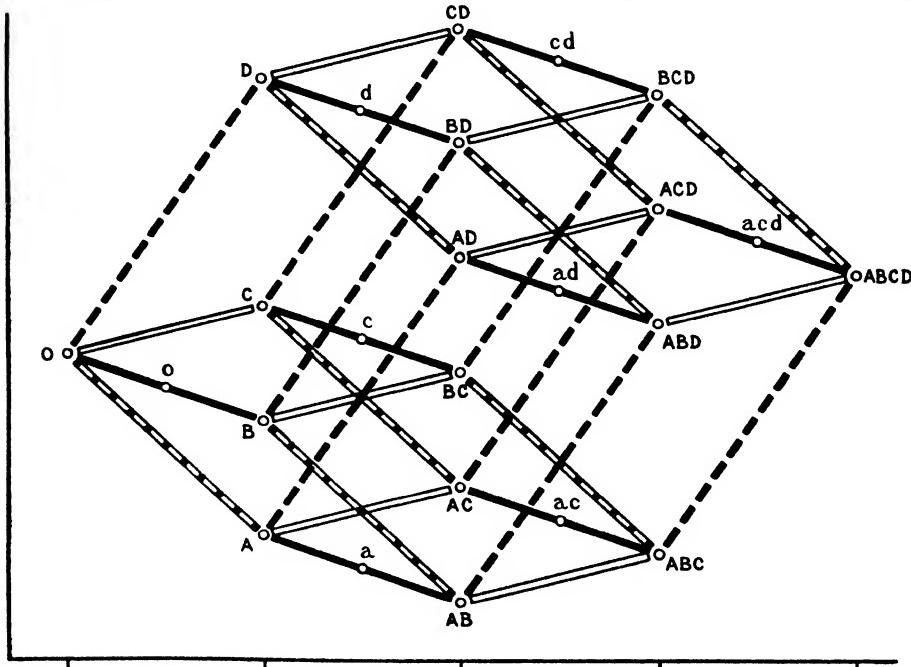
FIG. 2. Ideal diagram illustrating a hypothetical $2 \times 2 \times 2 \times 2$ experiment, without interactions. For full explanation see text.

one of these second-order systems may be identified geometrically by bisecting the eight lines of one particular type. If we designate the four factors A, B, C, and D, and determine the mid-points of the black lines (factor B) the new system of eight points may be treated exactly as those of Fig. 1 to give three main effects (A, C, and D) together with their three first-order interactions and one second-order effect. In Fig. 2 these points are shown as *o, a, c, d,* &c., these letters bearing the same interpretation in the simpler figure as O, A, C, D, &c., in the more complex.

By eliminating in this manner each in turn of the four factors the only information lost concerns the highest-order effect, and for the full presentation of this the complete diagram of Fig. 2 is necessary. A geometrical representation of the magnitude of this interaction may readily be made from a comparison of the second-order effects of any three factors at both levels of the fourth. Thus the sixteen points of Fig. 2 may be divided into two such sets of eight by grouping together those at the left extremities of the black lines, without factor B, and those at the right extremities, with it. From each set

may be derived (as in Fig. 1, *a*, *b*, A, B) four points which may be reduced to two by bisecting *a* B and A *b*. We are then left with two sets of two points each to represent the total third-order interaction, and these points stand in relation to this effect exactly as do *a*, *b*, A, B of Fig. 1 to the second-order effect.
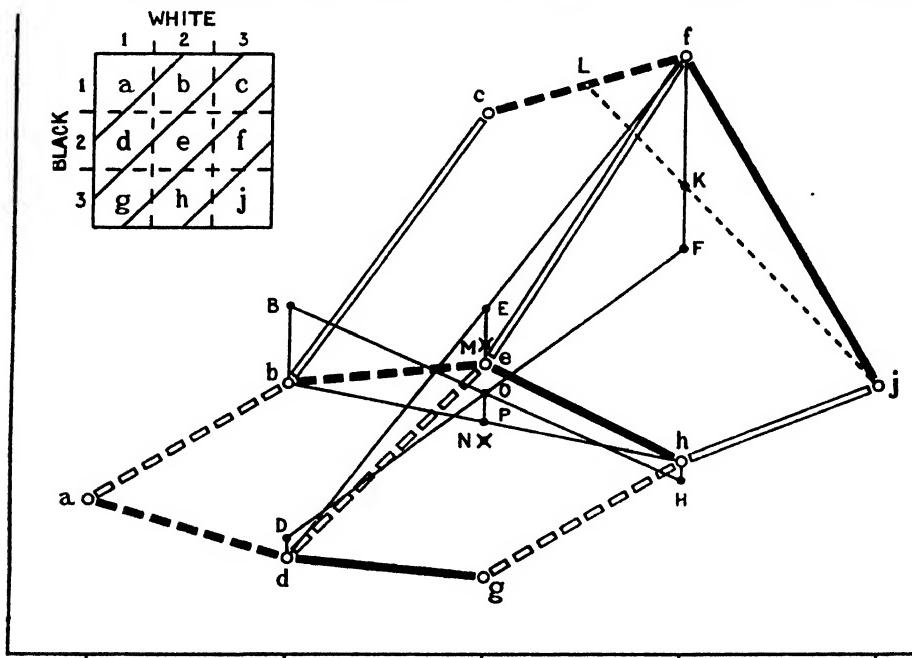
FIG. 3. Interaction diagram from a 3 × 3 experimental layout, illustrating the separation of individual degrees of freedom. For full explanation see text.

To obtain the final magnitude of the interaction it is only necessary once more to bisect the diagonals of the figure. In diagrams of the 2ⁿ type therefore an interaction of any order, *p*, may be obtained by bisection of the diagonals of a quadrilateral which itself represents two interactions, of order $p-1$, between the same experimental factors.

There remain to be considered experiments which include factors operating at more than two levels, and as a simple illustration a 3 × 3 layout may be taken. Fig. 3 illustrates the results of such an experiment, black lines referring as before to a change in level of one of the factors, and white to change of the other; moreover, a broken line refers always to a change from the first to the second level of the factor, while a continuous line indicates a change from the second to the third level. The manner of grouping the treatments along the abscissa will be obvious from the inset. Connecting the extreme left-hand point (lowest level of both factors) with the extreme right-hand point (highest level of both) there are six routes, each characterized by the fact that a broken line of either type is traversed before reaching a continuous one of the same type.

In such a diagram complete absence of interaction is found only when each of the small quadrilaterals constituting the figure is a parallelogram, hence the magnitude and kind of interaction may be rapidly assessed by the eye. In the previous examples each interaction represented only one degree of freedom, but here four degrees are involved, and if required the diagram serves readily for the demonstration of the magnitude associated with any of the four. Thus we may follow Fisher (1937, p. 137) and especially Yates (1937, p. 51) in their division of the total interaction effect:

1. The first degree of freedom may be regarded as representing a simple interaction between the *average* effects of the two factors, which appears in the diagram as the departure from a parallelogram of the quadrilateral defined by the points *acgj*. Bisecting the two diagonals of this figure in M and N we obtain $NM = \frac{1}{4}(a+j-c-g)$ as the first component.

2. The second degree of freedom is concerned with the difference between lines *abc* and *ghj* in their respective departures from rectilinearity. If we bisect the distance between *a* and *c* in B and that between *g* and *j* in H, then $Hh = \frac{1}{2}(g+j)-h$ and $Bb = \frac{1}{2}(a+c)-b$. The difference between these two, taking into account their directions, is therefore a measure of the component representing the second degree of freedom. It may be regarded as the departure of the lines BH *bh* and from parallelism, but in Yates's units (1937, p. 51) this departure must be measured directly as the algebraic difference in length of H*h* and B*b*, and not by the distance apart of the bisectors of the diagonals *b*H and B*h* of the trapezium *b*BH*H*.

3. The third is exactly analogous to the second, but concerns the other factor, being the difference between the black lines *adg* and *cfj* in their deviations from rectilinearity. As before, $Ff = \frac{1}{2}(c+j)-f$ and $Dd = \frac{1}{2}(a+g)-d$, and their difference—or the departure of DF and *df* from parallelism—measures this component of interaction.

4. The remainder of the interaction is made up in Yates's units of the difference between the sum of the departures from rectilinearity of lines *abc* and *ghj* and twice the departure of the line *def*; or equally as the corresponding difference between the sum of *adg* and *cfj* on the one hand and twice *beh* on the other. If O and P are defined as the points where BH and *bh* cross the central ordinate, then $OP = \frac{1}{2}(Bb+Hh) = \frac{1}{2}[\frac{1}{2}(a+c)-b+\frac{1}{2}(g+j)-h)]$. Also, if E is the mid-point of *df*, then $Ee = \frac{1}{2}(d+f)-e$. The difference between OP and E*e* therefore measures in Yates's units one-half the final component, and represents the fourth degree of freedom of the interaction. In taking the difference the directions of OP and E*e* must again be taken into account, measurements being taken from O to P and from E to *e*. If desired, of course, the difference in scale of the lines representing the fourth degree of freedom might be eliminated on the diagram in a variety of ways; for instance OP might be produced to P′, and *e*E to E′, making OP′ = 2OP and E′*e* = 2E*e*, reading the interaction component directly as OP′−E′*e*.

The diagram therefore makes possible not only the geometrical presentation

of the interaction as a whole but also that of its various components. The remaining statistical information to be derived from the experiment concerns the effect of each factor averaged over all levels of the other. For each factor this may be represented by three points; for the factor represented by black lines these points will be the means of (1) *a*, *d*, and *g*; (2) *b*, *e*, and *h*; and (3) *c*, *f*, and *j*. The points may be easily determined; thus for the mean of *c*, *f*, and *j* we may bisect *cf* in L and join L to *j*. The required point K is the intersection of L*j* with the ordinate of *f*. K*f* will of course be two-thirds F*f*. Similarly the other two points may be obtained. The two degrees of freedom associated with these three points for the one factor may then be readily discriminated and their relative importance assessed.

In experiments of the $n \times n$ type it will sometimes happen that the effects of both factors are in the same direction and of similar magnitudes. This leads to the crowding of the structural lines into a narrow strip, with loss of clarity. The author is indebted to Dr. F. Yates for the suggestion that in these circumstances one of the two factors may profitably be plotted in such a way that increasing dosage occurs from right to left. The distribution of treatments along the abscissa may then be obtained by drawing grouping lines at right angles to those shown in the inset of Fig. 3. The various groups will be (1) *g*; (2) *d*, *h*; (3) *a*, *e*, *j*; (4) *b*, *f*; and (5) *c*. In other ways the diagram is completed and interpreted just as is Fig. 3. This same figure may in fact be taken to illustrate the method by assuming that *g* represents the lowest level of both factors, and *c* the highest; the white factor is interpreted as before, but the black increases from *g* through *d* to *a*. Thus the lowest level of both factors and also the highest level of both appear on the central ordinate. This modification of the diagram is at first more confusing to use than the method of plotting both factors in the same direction, but may occasionally prove a better choice. Alternatively the method advocated previously may be used of plotting an increment in level of one factor to a different horizontal scale from an increment in level of the other. This method provides an unlimited choice, so that all sources of confusion in the lines of the diagram may be avoided. The scheme suggested by Dr. Yates is in fact a particular case of the more general method, in which the intervals chosen for the two factors are of equal magnitude but opposite in sign.

In order to illustrate the combination of several factors applied at different numbers of levels, a final example may be taken from a hypothetical $3 \times 2 \times 2$ experiment, requiring a diagram at about the practicable limit of complexity; in general more complicated experiments are better illustrated by multiple diagrams in the manner already described. The three levels of the one factor may be designated O, P1, and P2, and the higher levels of the remaining two as Q and R respectively. In Fig. 4 the difference between the lower two levels of factor P is represented by a broken black line and that between the second and third by a full black line. Differences of level of factors Q and R are indicated by white and pied lines respectively. In distributing the twelve

treatments along the abscissa the medium level of P (P1) is treated as factorially equivalent to the higher level of either Q or R, and the high level of P (P2) as equivalent to the combination in pairs of the higher levels of Q and R and the medium level of P. The framework consists of six white and six pied lines
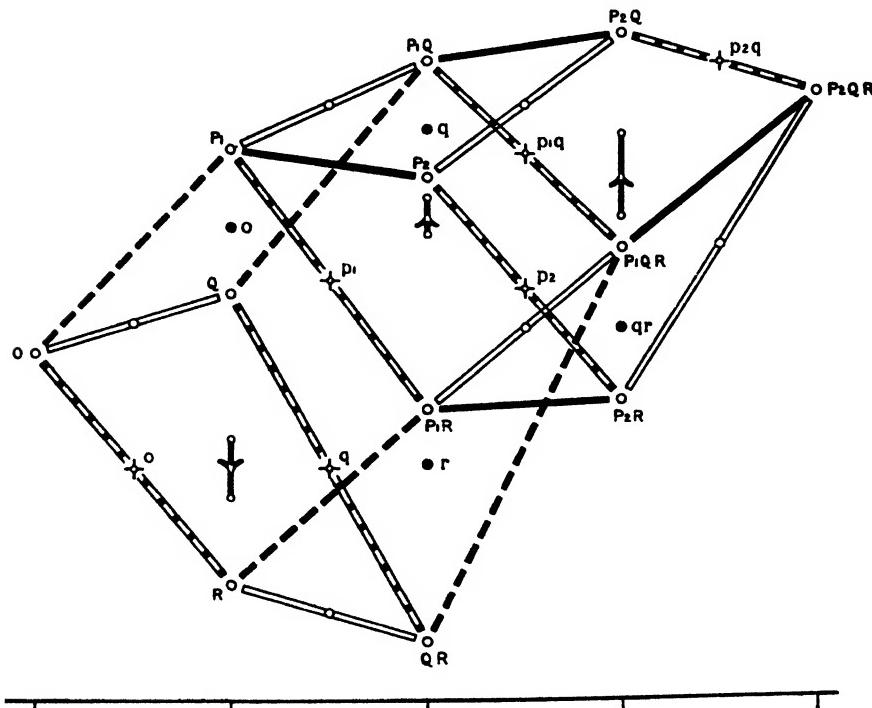
FIG. 4. Interaction diagram illustrating a 3 × 2 × 2 experimental layout.
For full explanation see text.

together with four each of the two varieties of black; it provides twelve alternative direct routes from O to P2 QR, each route traversing one of each type of line and the broken black prior to the continuous.

Of the interactions, that of Q with R alone represents only one degree of freedom. To derive it the mean values of treatments differing only in level of factor P are required. Four such sets are available, viz. O, P1, P2; R, P1R, P2R; &c. From each triplet, by the same method as was used to determine point K in Fig. 3, the mean point is derived. Thus point *r* is determined by treatments R, P1R, and P2R, and in the same way the black *points o, q,* and *qr* are found. The magnitude and kind of interaction appear as usual in the departure of these four points from a parallelogram.

For the interaction between P and Q we require the mean values of treatments differing only in level of factor R and these are given by the mid-points of the six pied lines. For a general view of the type and magnitude of the interaction we may note the departures from parallelogram form of the two

quadrilaterals defined by the points (marked with *crosses*) o, p_1, q, p_1q, and p_1, p_2, p_1q, p_2q, or perhaps more simply the departures from parallelism of the three lines joining o to q, p_1 to p_1q, and p_2 to p_2q. If desired the two degrees of freedom of the interaction may be separately represented. The first is concerned with the average effect of factor P and is measured by the departure of the points o, q, p_2, and p_2q from a parallelogram, this being accomplished geometrically by the usual device of bisecting the diagonals joining o to p_2q and q to p_2. The second degree of freedom is measured by the difference in the departures from rectilinearity of the lines obtained by joining on the one hand o, p_1, and p_2, and on the other q, p_1q, and p_2q. These departures may be represented precisely as in Fig. 3 by two vertical lines having p_1 and p_1q respectively as their upper extremities. The remaining first-order interaction, $P \times R$, is represented and analysed in the same way as $P \times Q$, using the mid-points of the six white lines.

The interaction between all three factors is perhaps most easily observed from a comparison of the three quadrilaterals representing the $Q \times R$ interactions at all levels of P. The three vertical black lines represent these interactions just as do the corresponding lines in Fig. 1, the direction of the arrow-heads distinguishing between positive and negative differences. In an analogous manner to the first-order interactions, the two degrees of freedom of the second-order are represented in magnitude by (1) half the difference in length, taking into account their algebraic signs, of the two lines on the ordinates of single and triple factors; regarding these as opposite sides of a quadrilateral, the required magnitude is obtained geometrically by bisection of the diagonals as usual; and (2) the difference in length between the line on the ordinate of double factors and the mean of the lines on the ordinates of single and triple factors. If desired this latter algebraic mean may itself be inserted on the ordinate of double factors by noting the intersections on the latter of lines joining the appropriate ends of the two lines on the single and triple factor ordinates respectively.

Finally, as was the case with Fig. 1, the mid-points of the lines representing the second-order interaction (marked by the tips of the arrow-heads) give the mean values over all other treatments at the three levels of factor P; the separation of the two degrees of freedom involved is carried out in the manner described for similar separations. If the mean effects of factors Q and R over all levels of P are required they may of course be obtained by the bisection of opposite sides of the quadrilateral defined by the black *points o, q, r, qr.*

It may perhaps be pointed out that the sole novelty of the methods outlined in this paper lies in the general scheme of diagrammatically representing in one plane the results of a complex experiment, and of geometrically representing the interaction effects of various orders. In this way the major effects of the factors immediately become apparent to the eye, and others are readily made so. For estimations of statistical significance the established algebraic methods of analysis of variance will naturally be used.

SUMMARY

A type of diagram is described that enables the results of any experiment of factorial design, including the various interaction effects, to be presented fully and concisely in one plane. Examples of diagrams illustrating the $2 \times 2 \times 2$, $2 \times 2 \times 2 \times 2$, 3×3, and $3 \times 2 \times 2$ layouts are given and discussed.

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Physiological Studies in Plant Nutrition

XI. The Effect on Growth of Rubidium with low Potassium Supply, and Modification of this Effect by other Nutrients

PART I. *The Effect on Total Dry Weight*

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With eight Figures in the Text

INTRODUCTION

INVESTIGATIONS on the physiological effects of potassium deficiency in barley have been carried out at this Institute over a number of years. The original nutrient solution used had a high sodium and low calcium content, and large-scale growth experiments were at first undertaken to discover the principal interaction effects between potassium and nitrogen (Mathur, 1933) and between potassium and phosphorus (Verma, 1935). Other lines of investigation included observations of respiration rate, and nitrogen and carbohydrate metabolism on several selected treatments from the more extensive growth experiments (Gregory and Sen, 1937; Richards, 1938). These experiments revealed consistently many results in relation to potassium nutrition which differed radically from previous results described in the literature, and it thus became apparent that potassium effects depend to a surprising extent upon the

specific nature of the nutrient solution, and particularly upon the levels at which the calcium and sodium supplies are maintained. Further investigations were therefore undertaken to study more directly these complex interactions, the most comprehensive experiment being that of Shih (1934), who investigated potassium deficiency at various levels of the sodium-calcium ratio and at two phosphorus levels. The water-content data from this experiment illustrate the complexity of the interactions involved (Richards and Shih, 1940).

Having investigated the effects of varying potassium supply at high levels of sodium and low levels of calcium, at high levels of calcium in the absence of sodium, and also at high levels of both factors, further advance necessitated a study at low levels of both elements. In practice this required the replacement of sodium and calcium salts by ammonium nitrate and ammonium phosphate to supply the principal nutrient elements, and this unfortunately superimposed upon the calcium-sodium interactions under investigation other differential effects due to the replacement of much of the nitrate by ammonium ion. Except for the ammonium ion therefore, and so far as metabolism is concerned, ammonium may be regarded as practically equivalent to nitrate, this solution when used at low potassium levels is generally deficient in cations, which comprise only small quantities of calcium and magnesium. In such a solution at high potassium levels barley grows fairly successfully, though not so well as when sodium or calcium is maintained at a higher level, and ammonium is absent. But the plants so grown are very sensitive indeed to potassium starvation, and at very low levels of this element extensive disorganization occurs at a very early age. This disorganization does not appear to be appreciably dependent on the acidity of the medium. Characteristics of these plants will be described later; it is sufficient here to state that growth almost ceases for a considerable period at the first or second leaf stage, and that a large proportion of the plants die during this time.

In recent years similar disastrous effects of potassium deficiency in solutions containing ammonium salts have been described, in particular by Turtschin (1934), Schropp and Arenz (1939), and Wall (1940), and it is claimed that the observed toxicity is a direct consequence of the accumulation of excess ammonium within the plant in the absence of potassium. It was obviously desirable to discover so far as possible the immediate cause of the failure in the present experiments, and in a preliminary survey the possibility was explored of other alkali metals being able to perform to any extent the function of the missing potassium and so enable the plants to grow. To aliquots of the low potassium solution there was therefore added, in amounts equivalent to one-third the normal potassium level, the sulphate of one of the following elements: lithium, sodium, rubidium, caesium. Lithium, sodium, and caesium were completely ineffective; in fact in the presence of any one of these, injury appeared to be more pronounced than in its absence. With rubidium the effect was entirely different, and the element proved to be little less effective

than potassium in removing the immediate toxic symptoms and allowing early growth to proceed. Since sodium is worse than useless in this connexion, it is clearly not a matter of merely providing a harmless cation, but of an effect specific to potassium and rubidium. The plants with rubidium developed after a time curious and highly characteristic symptoms, to be described later, and eventually most of them died, owing largely to direct toxic effects attributable to rubidium itself. This was confirmed by treating with similar quantities of rubidium plants growing in potassium deficient solutions free from ammonia and at high sodium or calcium levels; in all cases similar symptoms and toxic effects eventually developed.

The beneficial effect of rubidium on early growth being established, there remained to determine the optimum level of the element so far as total growth is concerned. Under the conditions of the experiment this proved to be approximately one-sixth gm. Rb_2O per pot of three plants, i.e. the equivalent of about one-twelfth of the standard dose of potassium for optimal growth. With this amount of rubidium surprisingly good growth occurred. In the initial stages of growth increasing response to rubidium was found up to four times this dosage, though from a very early period plants grown at the higher concentrations were abnormal in appearance. Their high early growth rate soon began to decline and eventually maximum response shifted to the dosage stated, where it remained until harvest.

Following on the successful use of rubidium with potassium deficient, high ammonium solutions, a wider survey was made of its effect on several of the low potassium nutrient types used by Shih (1934). The survey revealed a striking interaction in relation to the rubidium effect between the ammonium-calcium status of the nutrient solution and the phosphorus level. In consequence a large-scale growth experiment was set up to investigate this interaction more thoroughly, the primary aims being to throw further light on (1) the immediate causes of the early injurious effects noted in high ammonium, low potassium solutions, and (2) the relationship of the observed rubidium effects to the corresponding effects of potassium. The present paper deals with the data from this experiment, though reference is made to results of other experiments having a bearing on these issues.

The fact that potassium is an essential element in the growth of higher plants was early established by the use of culture solutions. The experiments of Lucanus (1865), Birner and Lucanus (1866), and Loew (1878) demonstrated that none of the related elements lithium, sodium, rubidium, and caesium can be successfully substituted for it in the nutrition of phanerogams, and these findings have been amply confirmed by later workers (see Alten and Gottwick, 1933, and Eckstein, 1935). But it has been claimed that rubidium may largely if not entirely replace potassium in some lower plants. Thus Stanbury (1934) states that if the diatom *Nitzschia closterium* be supplied with small amounts of rubidium growth is equally good as with equivalent amounts of potassium. In the rubidium cultures growth is then limited to an increase

of about ten times the original number of cells; in larger amounts rubidium is harmful, though equivalent amounts of potassium give considerable further growth. Pirson (1939) on the contrary claims that rubidium may completely replace potassium in cultures of *Chlorella*, though growth and photosynthesis are slower than in potassium cultures. If photosynthesis is inhibited by potassium deficiency, the addition of potassium leads to recovery which, according to Pirson, occurs in two phases: an increase during the first few hours, representing a direct action of the element on the photosynthetic mechanism, followed by a further rise accompanying cell reorganization, and the elaboration of proteins, chlorophyll, &c. In the primary phase of recovery potassium may be completely replaced by rubidium and partly by caesium; in the secondary rise rubidium is not so effective as potassium, while caesium is not only ineffective but leads to inhibition. Sodium and lithium do not increase the assimilation rate of potassium deficient cells. In deficient cultures again the rate of respiration is increased; application of potassium or rubidium reduces this to some extent, and caesium even more so. The suggestion is made that the effects of the various alkali metals on the immediate recovery of photosynthesis may be related to their ionic mobilities.

Even with higher plants increases in growth due to rubidium have occasionally been noted, observations which have not so far received the attention they deserve. In particular, Scharrer and Schropp (1933) in a single experiment with maize effectively demonstrated that during the vegetative period increases in yield up to 50 per cent. could be obtained in the presence of potassium by the addition of rubidium over a wide range of concentrations. Nevertheless these workers were content merely to put this result on record and then proceed to demonstrate once more that if the potassium of the culture solution is partially replaced by rubidium crop yield diminishes, while if it is wholly replaced the plants die. In the investigation of the physiological role of any one element the discovery of a related second element which may completely replace the normal one will probably be of limited interest, just as would be the discovery that a related element cannot at all be used as a substitute. But if a second element can be found which provides satisfactory partial substitution, then experimental separation of the similar and dissimilar effects of the two elements may prove a valuable aid in the analysis of the metabolic effects of the essential element. It is clear that rubidium can perform some necessary function almost if not quite as successfully as does potassium; therefore in considering the physiological effects of rubidium emphasis may well be diverted from the impossibility of complete substitution for potassium in higher plants to the much more interesting consideration that part of the total function of potassium may apparently be successfully performed by the heavier element. In this way the analysis of the function of potassium may perhaps be carried further, a possibility which in the past has been largely disregarded.

It has been noted by Arndt (1922) and Heller *et alia* (1934) that the adverse

effects of rubidium application are reduced by higher potassium levels, less rubidium being absorbed under these conditions, and Hurd-Karrer (1939) has stressed this relationship of the two elements in illustration of her theory of 'mass-antagonism'. In her investigations possible beneficial effects of rubidium, as observed by Scharrer and Schropp, were ignored. She also states that rubidium toxicity was investigated with reference to phosphorus and calcium levels, and that only negative results were obtained. In the present paper very large differences will be described in the effects of rubidium due to changes of both phosphorus and calcium supply.

The causes of the detrimental effect of rubidium are not known; indeed it has been doubted whether the observed toxicity is a positive effect. Thus Arndt (1922) believed the effect was unlikely to be due so much to a specific toxicity of the element itself as to displacement of potassium and interference with the essential functions of that element. In this view he has been supported by Blanck *et alia* (1933). It is extremely doubtful whether the thesis of Arndt can be maintained. Simple interference with potassium might reasonably be supposed to do little more than bring about or accentuate symptoms associated with simple potassium deficiency; this is very far indeed from being the case. In none of the various nutritional series to be described in the present paper does the addition of rubidium to plants at a higher potassium level lead to the development of symptoms at all resembling those of the plants without rubidium at a lower potassium level; on the contrary all finally tend towards the type to be described as characterizing excess rubidium. The symptoms of potassium deficiency are very different in the various series, and if Arndt's conclusion is correct rubidium addition at a higher potassium level might be expected to lead to greater diversity in appearance among the treatments; in fact the addition results in a notable convergence towards a completely new and abnormal type. Hence the development of these particular symptoms must presumably be ascribed to a specific toxic effect of rubidium, and in the present paper it is so interpreted.

EXPERIMENTAL METHODS

Barley (Plumage Archer) was used with three plants per pot in sand culture out of doors in the usual way. Seed was sown on May 4, 1939. Three main types of solution were investigated, one being the high calcium type used by Shih, in which the nitrogen and phosphorus were given entirely as calcium salts. In another the calcium phosphate was replaced by its equivalent of ammonium phosphate, and the nitrogen level made up to that of the high calcium solution with ammonium nitrate. The third type of solution was intermediate in composition between these, a half-quantity of each of the four main salts being used. The two extreme types were combined with two levels of phosphorus, one ten times as high as the other; the intermediate type was investigated only at the high phosphorus level, i.e. the level used by Richards and Shih (1940), and designated by them H. Potassium was supplied at two

levels, namely one-ninth and one-eighty-first respectively of the normal requirement; these are the two levels designated K3 and K5 in previous work. It should be emphasized that all solutions used were thus deficient in potassium. All these nutrient solutions were employed both in the absence and presence of rubidium, constituting a total of twenty treatments. As the difference between the two potassium levels approximates to the equivalent of the optimum rubidium dose found in previous work, the rubidium supply actually used was made exactly equivalent to this difference, so that plants at the low potassium level supplied with rubidium were presented with the same total of alkali metal as plants at the high potassium level without rubidium.

Nomenclature.

To simplify the symbols used for the treatments the nomenclature in this paper has been changed from that previously in use. The *high* level of phosphorus will be designated P, that of potassium K, and the presence of rubidium R. Low levels of phosphorus and of potassium and the absence of rubidium will in general receive no symbol, the exception being the simultaneous occurrence of all three conditions, which will be designated O. These three variables lead to eight nutrient combinations, which therefore receive the symbols O, P, K, R, PK, PR, KR, and PKR respectively. These are again combined with each of the three main nutrient types. The high calcium series will be designated C, the high ammonium series M, and the intermediate series X. Thus the three treatments receiving high phosphorus and low potassium in the presence of rubidium will finally receive the symbols C : PR, M : PR, and X : PR respectively. The symbols for all treatments are entered below in Table I.

<div align="center">TABLE I</div>

<div align="center">*Nomenclature*</div>

	High ammonium.		High calcium.		Intermediate ammonium and calcium.
	Low phosphorus.	High phosphorus.	Low phosphorus.	High phosphorus.	High phosphorus.
Low potassium	M : O	M : P	C : O	C : P	X : P
High potassium	M : K	M : PK	C : K	C : PK	X : PK
Low potassium + rubidium	M : R	M : PR	C : R	C : PR	X : PR
High potassium + rubidium	M : KR	M : PKR	C : KR	C : PKR	X : PKR

Nutrient Solutions.

The actual amounts of the salts used for the various treatments are given in Table II; in addition the usual ferric chloride and manganese sulphate were applied. The pH of all solutions was adjusted by means of sulphuric acid to that of treatment C : P.

TABLE II

Nutritional Scheme

Basal nutrients (gm. per pot)

	M : O	M : P	C : O	C : P	X : P
NH_4NO_3	3·61	3·61	—	—	1·805
$(NH_4)_2HPO_4$	0·111	1·11	—	—	0·555
$(NH_4)_2SO_4$	1·00	—	—	—	—
$Ca(NO_3)_2·4H_2O$	—	—	12·65	12·65	6·33
$CaH_4(PO_4)_2·H_2O$	—	—	0·106	1·06	0·53
$CaSO_4$	—	—	0·51	—	—
$CaCl_2·6H_2O$	0·37	0·37	0·37	0·37	0·37
$MgSO_4·7H_2O$	1·25	1·25	1·25	1·25	1·25

Potassium and Rubidium (gm. per pot)

	O	K	R	KR
K_2SO_4	0·023	0·206	0·023	0·206
Rb_2SO_4	—	—	0·279	0·279

Sampling.

To each treatment were allotted fourteen pots, of which three replicates were removed at each of three fortnightly sampling periods during growth, leaving the remaining five for a final sample after ample opportunity had been given for ripening. Owing to the amount of material to be dealt with each fortnightly sample extended over three days, one pot from each treatment being sampled every day; these sampling periods were June 12–14 (30–32 days from germination), June 26–28, and July 10–12. The final larger sample was extended over a considerable time corresponding to the ripening behaviour under the various treatments. The dates of harvest for the individual treatments are given in Table III.

TABLE III

Harvesting dates, 1939

	C : P	C : O	M : P	M : O	X : P
O	Nov. 8	Sept. 14	Oct. 24	Oct. 24	Nov. 6
K	Sept. 15	Sept. 14	Oct. 24	Sept. 15	Oct. 25
R	Nov. 8	Nov. 8	Nov. 6	Nov. 7	Nov. 6
KR	Sept. 15	Oct. 23	Sept. 15	Sept. 14	Sept. 14

At each of the three samples during the growth period the data collected included fresh and dry weights of green leaf blades and stems (including leaf bases and unexpanded leaf still within the previous sheath), dry weights of roots and dead leaf material, tiller numbers, and measurements of the length and breadth of every leaf. Although it was impossible in the time available to determine actual leaf areas, estimates of these have been made from the leaf measurements. For this, factors derived from previous work, relating area to the product of length and maximum breadth, have been used. Most of these factors were obtained directly from observations of similarly treated

plants in the previous year or from those of Shih's experiment, but for a few treatments no direct estimates were available. This was the case for the X series; since these treatments were intermediate between those designated M and C, and the behaviour of the plants was also intermediate, the mean factors from the corresponding treatments of these two series were used. Actually variation among the treatment factors is comparatively small; greater variation is found within any one treatment with age. Errors in estimations of net assimilation rate due to the use of incorrect factors must be small relative to the errors of sampling.

Besides the tiller number data obtained from the pots used at each sampling period, frequent counts were taken on all the remaining pots in the field during the growth cycle. Data collected at the final harvest include height of plants, number of shoots, ears, and fertile grain, and weight of ears, grain, stems, and roots.

The collected material was subsequently ground and bottled; and since it became obvious that phosphorus level had a very great effect on the response of the barley to rubidium, some of the material was later ashed at about 300° C. and the total phosphorus content estimated. The method of estimation used was substantially that of Holman and Pollard (1937), the intensity of the colour developed being estimated in a Bausch and Lomb colorimeter by comparison with standards prepared at the same time as the samples. Phosphorus estimations were made on all fractions (leaf, stem, root, and dead matter) separated at sample 3, so that for all treatments the total uptake at this time is known, and also on the green leaf fraction of sample 1. At sample 3 differences due to treatment were similar in all fractions; moreover, treatment effects on the green leaves at samples 1 and 3 were also closely similar, hence it was considered unnecessary to estimate the phosphorus in the remaining fractions of sample 1 and in those of sample 2.

The present paper is confined to a description of the main visual characteristics developed under the various treatments, and to the presentation and elucidation of the total dry weight data; this necessitates the presentation of the phosphorus analytical results.

EXPERIMENTAL RESULTS

A. *General Characteristics*

Symptoms associated with excess rubidium. Excess of rubidium leads usually to premature death, the symptoms being similar for all types of nutrients investigated, i.e. high ammonium, high calcium, or high sodium; earlier symptoms may vary with the general type of solution, and particularly with phosphorus level. At high phosphorus levels in general the resulting plant has at first short, thick roots and intensely blue-green, waxy leaves. The leaves are quite characteristic in other ways, being very wide with prominent mid-ribs; they are curiously brittle, and the spiral twist normal to the barley

leaf is much exaggerated. After a few weeks the characteristics of the new parts change abruptly and strikingly. Leaves are now produced with very little chlorophyll and having a light grey-green colour; torsion becomes much less obvious and the laminae far from being unusually wide are now exceedingly narrow. Tillering becomes excessive, and eventually one such plant may produce well over a hundred minute tillers. At the time of the transition it is usual to find plants whose older leaves are dark blue-green, wide, and twisted, while the newer ones are of the later type; leaves intermediate between the two types are only rarely found. Eventually, when all early leaves are dead the plant remains dwarf and very bushy, having an excessive number of thin, weak tillers bearing very short and narrow light-grey leaves; the appearance casually is somewhat like that of a group of thrift. No earing occurs and many of the plants never reach this stage, dying earlier.

Symptoms occurring in the main experiment. In the main experiment a rubidium level approximating the optimum was used so that the extreme type described above was absent. Nevertheless characteristic effects of rubidium appeared, and at the high phosphorus level most of the plants receiving the element eventually developed symptoms similar to, though much slighter than those finally characteristic of excess rubidium.

Among the treatments, colour and other differences were becoming pronounced by the beginning of June. Already treatments M : O and M : P were the most yellow and of poor appearance, M : O being slightly the better of the two. At this early stage and for some time onwards both series almost ceased growing, and the leaves already produced began to die off rapidly. A considerable number of the plants in both died completely during the next few weeks. Active growth in the remainder was generally resumed about the middle of July, and with its resumption toxic symptoms disappeared and the colour became a more normal green. Stem elongation followed, but tillering was restricted in both series and almost completely absent in M : P. The base of the stem was frequently too weak to support the elongating structure, leading to collapse. Response occurred at one of the later nodes which enlarged and assumed a right-angled bend so that the stem above this point again became upright. A second fall sometimes followed, and this might be succeeded by further recovery at another node, in which case the axis of the stem became tortuous.

A curious feature was observed in two plants from the high ammonium series at very low potassium levels, one in 1938 and one in 1939. In both instances the plant, until stem elongation was complete, consisted only of a main axis, and in both a very late tiller was produced, that of 1938 being in the axil of the ninth (penultimate) leaf and that of 1939 in the axil of the tenth (last) leaf, whereas in barley tillering normally ceases after the sixth leaf. In the former instance the tiller produced five leaves followed by an ear, and in the latter two very minute leaves up to the time of harvesting, on October 24. The main axis above the point of insertion of the tiller was in both plants

much reduced and distorted, but the terminal ear was large and well developed. The axis of the tiller too was much reduced and twisted, so that the appearance of the 1938 plant was rather that of one stem bearing two ears than of two distinct tillers. In both instances again the lower part of the main axis was tortuous, having two right-angled bends. It is impossible to state whether the production of tillers in such unusual positions was a direct consequence of the nutritive conditions involved or an indirect result due to the crumpling of the axis interfering with normal translocatory movements.

The addition of potassium to one-ninth of the standard amount (treatments M : K and M : PK) overcame the early toxic symptoms and the plants grew reasonably well, being the best of the M series until after the second sample. Thereafter the leaves proceeded to die off rapidly, beginning very characteristically with those of *medium* age, and in treatment M : PK became closely speckled with dark brown spots, simulating to some extent a severe attack of rust; this symptom is characteristic of potassium deficiency under these conditions. At the same time assimilation rate in this treatment fell to a very low level, so that growth was considerably retarded. By harvest their condition was extremely poor, most of the stems and ears having died prematurely rather than ripened. In many plants a second cycle of tillers was growing at the time of harvesting. Plants from treatment M : K eventually exhibited symptoms associated with phosphorus deficiency, especially after shooting, the tall stems having a blue waxy appearance with considerable reddish colouring on the grain and awns.

The effect of addition of rubidium to the treatments M : P and M : O was most striking, since it seemingly overcame completely the toxicity characteristic of the early stages of these plants. The rubidium level chosen approximated to the most favourable level found in the previous year, and though early growth was not so rapid as would have occurred had a higher level been used, yet the total growth made was considerably greater, since the later adverse effects of excess rubidium were largely avoided. In earlier stages the plants were dark green with broad leaves showing an abnormal amount of twisting, but these symptoms were developed to only a slight extent. The typical later symptoms associated with rubidium application were more pronounced: short, thick roots, excessive tillering, narrow and short grey-green leaves. After the stems had 'shot', at the time of ear emergence, all the tillers died and a second cycle was produced from their bases. This second crop eventually 'shot' and produced green ears before the plants were finally cut; in some instances new tillers were at this time still being produced from the base. Greater improvement was effected by the addition of rubidium to treatment M : P than to M : O; plants from treatment M : PR were nearly as large as those from M : PK, though the type of growth was entirely different.

Improvement in general condition also followed the addition of rubidium to treatments M : PK and M : K, leading to a slower rate of dying of older leaves. In treatment M : PKR effects of rubidium were not obvious at first,

and in fact up to the second sample the plants lagged slightly behind their M : PK controls; but for some time afterwards energetic growth was made in contrast to the rapid dying of old parts in the control treatment. At harvest, plants from the two treatments were not dissimilar in general appearance, those with rubidium being much larger, with more tillers, ears, and grain, while their later leaves had been distinctly narrower. At the lower phosphorus level the improvement in appearance was not reflected in total growth; in fact the growth curve for treatment M : KR was consistently slightly below that of M : K. Rubidium addition here led to rather earlier ripening, and finally to a type of plant very similar to that of M : PKR, rather taller, but smaller and less bushy; in earlier stages typical phosphorus-deficiency colouring developed to some extent.

In the high calcium series at the higher phosphorus and potassium levels (treatment C : PK) good early growth was made and dark green leaves produced. By the first sample a few small brown spots had appeared on older leaves; about the time of maximal leaf area all leaves were similarly speckled, though not so severely as in treatment M : PK with high ammonium. The last leaves were free from these symptoms. Speckling also occurred at a later stage in treatment C : P, where it was preceded by the appearance of light, almost white patches. These were irregularly distributed over the leaves, and locally the cells had completely lost their chlorophyll, though they remained alive for some time. Eventually the patches died and dried out, leaving the other parts of the leaves normal in appearance. By the beginning of September there was still much brown speckling on the older leaves in treatment C : PK and some in C : P. In the former many stems had collapsed but there was less dead matter than in treatment M : PK and tillering was continuing; while in treatment C : P the brown spots were rapidly disappearing and the plants were generally of good colour with the older leaves yellowing.

The addition of rubidium to these two treatments led in the earlier stages to no very striking changes; during the period of the samples treatment C : PR was little if any better than C : P, while C : PKR lagged behind C : PK. In both sets a certain amount of brown speckling occurred, particularly on the older parts of the leaves. During the latter half of July and in August the growth rates of the two rubidium treatments were considerably greater than those of their controls, so that eventually the beneficial effects of the element were very obvious. Thus by September treatment C : PKR was considerably bigger and better than C : PK; there were few dead ears and little dead matter altogether, while the straw was strong and upright. At this time the plants in treatment C : PR were waxy blue-green in appearance, with some reddening. The tillers were strong and upright, though thin; older ears were dead with no swollen grain, and there were many new tillers. Although the five pots carried to harvest produced over 150 ears, only one fully swollen grain was found.

Reduction of phosphorus level induced pronounced symptoms associated

with phosphorus deficiency in treatments C : O and C : K. By the beginning of June a purpling of the leaf-tips and purple veins on the sheaths were noticeable in both series, and these rapidly became more marked, particularly at the low potassium level. Some traces of the white patches and brown speckling characteristic of the corresponding high phosphorus series were also found. Tillering was limited but eventually good ears were formed, these treatments producing the highest ear : straw ratios of the experiment.

The addition of rubidium here produced great and generally adverse effects. By the beginning of June treatment C : R had developed an intense purple in the leaves and down the veins of the sheaths, and by the time of the first sample the whole of the leaf bases and first leaf, and the tips of other leaves, had become a rich, brilliant red. Growth was slow and in habit the plants were stiff and erect, with a minimum of dead matter. Tillering scarcely began until towards August, when it increased somewhat; not until the last week of that month did the tiller number reach that of treatment C : O, in which it was also very low. At harvest only three of the surviving fourteen plants had more than four tillers each; one of these produced fourteen, almost all of which were very dwarf new shoots displaying the more usual characteristics of rubidium treatment at this time, notably short narrow leaves of light grey-green colour. The majority of the plants developed these symptoms to a slight extent only and there was considerable reddening to the end. In size, treatment C : R remained throughout much smaller than C : O. At harvest fertile grain was completely absent. The relationship of treatment ·C : KR to C : K was similar to that of C : R to C : O, and the symptoms within the treatment C : KR everywhere resembled those of C : R, though never so extreme. Although fertile grain was produced by treatment C : KR, it was considerably smaller in amount than in C : K. The symptoms induced by rubidium in high calcium treatments at low phosphorus and potassium levels are strikingly like those usually associated with phosphorus deficiency, but are greatly intensified.

The calcium-ammonium treatments X : P and X : PK exhibited symptoms generally intermediate between those of the corresponding C and M treatments. In X : P rapid death of leaves was already occurring at the first sample, the leaves dying to a characteristic cream-buff colour. Some white patches and brown speckling appeared later. By September these symptoms were disappearing; the younger leaves generally were a good green, while older ones had turned bright yellow. Treatment X : PK began very well and not until the third sample were there serious signs of deterioration. By September they were in very poor condition, rather like M : PK, but with less dead matter; many stems were broken and new tillers were being produced. At harvest they were extremely poor, many having died prematurely. The stems were excessively weak, almost all having collapsed and broken in several places, the ears frequently becoming detached.

Addition of rubidium to treatment X : P was beneficial to vegetative growth

throughout life. Adverse symptoms were much slighter and the plants were doubled in size. Tillering rate was considerably increased throughout the growth cycle. By September the general appearance was similar to that of treatment M : PR, the plants being rather greener with a purplish colour on many sheaths. Later there was more deterioration, and ripening did not occur; some of the plants were practically dead by the time of harvesting, and although with rubidium there were many more ears practically no grain was set, as was the case also in the C : PR treatment. The total weight at harvest was, however, actually greater than that of treatment X : PK. Just as with the high phosphorus and potassium treatments of both series M and C, the effect of rubidium addition to treatment X : PK was not obviously beneficial until the third sample, growth rate being distinctly reduced in the early stages. The ultimate effects were again decidedly beneficial; vegetative growth continued unimpaired long after that of treatment X : PK was failing, so that the final yield was considerably more than doubled. The improvement appeared to be quite general, and the plants ripened normally. The ear : straw ratio was much higher than in treatment X : PK and nearly twenty times as many fertile grains were produced, though the average weight per grain was somewhat reduced.

B. *Total Dry Weight*

Total dry weights at the three sampling times and at harvest are presented in Table IV and for the series M and C as interaction diagrams in Figs. 1–8. For a full description of the use and interpretation of such diagrams reference may be made to the preceding article in this journal (Richards, 1941).

The sixteen treatments from the M and C series provide a 2^4 symmetrical table. For simplicity the diagrams treat separately the data of the two series, each diagram presenting the results of a 2^3 factorial experiment at one sampling time. The eight treatments are grouped according to the number of experimental nutrient factors they have received at the higher level, and thus fall into four groups which are spaced at equal intervals along the abscissa. Yields are plotted above these positions in the usual way. The eight treatment points are interconnected by a network of twelve lines, three sets of four being distinguishable: a black line joining two treatments indicates a difference of phosphorus level between them, a white line a difference of potassium level, and a pied (black and white) line one of rubidium application. The diagram so constructed contains six quadrilaterals, each representing the interaction of two of the factors at one level of the third. The kind and degree of interaction may be immediately seen in the manner and extent to which the quadrilateral departs from a parallelogram. The mid-points of the twelve constructional lines are also indicated. The mid-point of a black line clearly gives the mean value of two treatments differing only in phosphorus level; hence the departure of the mid-points of the four black lines from parallelogram

form represents the total first-order interaction between potassium and rubidium. The other two first-order interactions are similarly obtained from the mid-points of the potassium and rubidium lines respectively.

The absolute magnitude of the interaction between potassium and rubidium
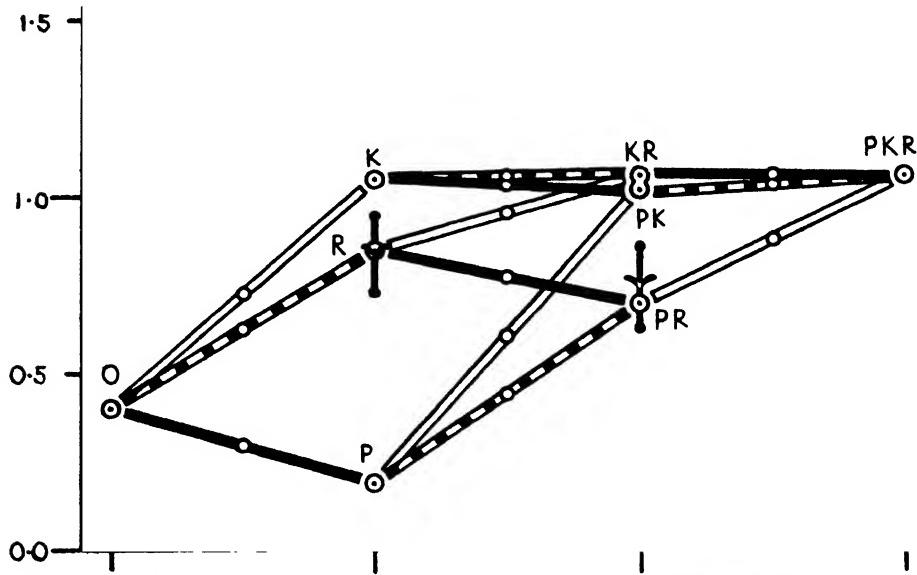
FIG. 1. Interaction diagram for total dry weight (gm. per pot) of series M, sample 1. Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application.

at the low phosphorus level is given by $\frac{1}{2}(KR+O)-\frac{1}{2}(K+R)$. The two portions of this expression may be easily determined on the diagram by bisection of the two diagonals of the quadrilateral defined by the positions of the four treatment points concerned. One of the two vertical lines on each figure joins these two bisectors, and the length of the line gives directly the magnitude of the interaction. The arrow-head on the line defines the algebraic sign of the interaction, a positive value being shown as an upward direction. The second orientated vertical line similarly represents the interaction between potassium and rubidium at the high phosphorus level. The interaction of triple factors now appears as the departure from parallelism of the two lines which may be drawn joining corresponding ends of the interaction lines, and is given in magnitude by half the difference in length of these two latter, taking into account their algebraic signs. Finally, the tips of the arrow-heads also serve to indicate the mid-points of the two interaction lines. These give respectively the mean values from the two sets of treatments O, K, R, KR, and P, PK, PR, PKR; hence the slope of a line joining the arrow-heads represents the mean phosphorus effect over the experiment.

While for reasons of space the statistical examination of the growth data is

not presented it may be pointed out that throughout the remainder of the paper only those results are considered which are shown to be highly significant by the methods of the analysis of variance, and are presumably real. However, the degree of variability of the individual treatments was very
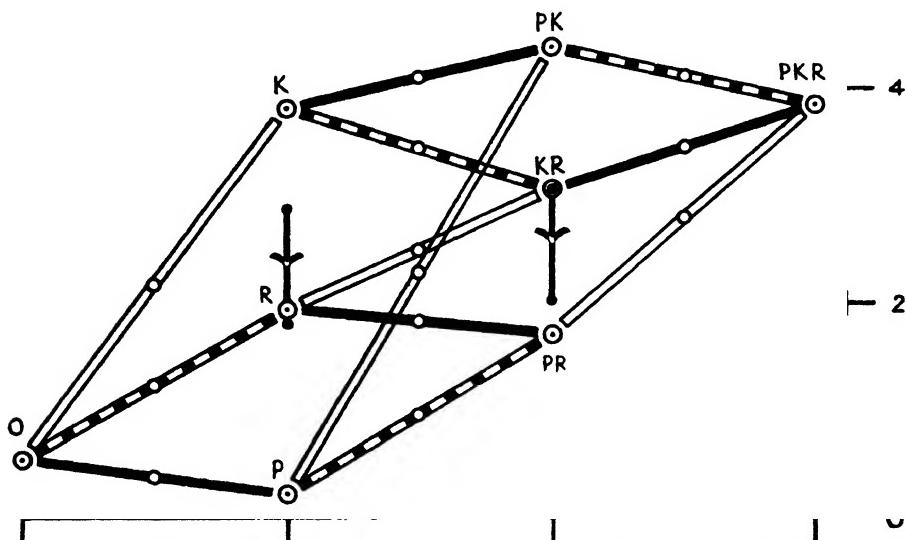
FIG. 2. Interaction diagram for total dry weight (gm. per pot) of series M, sample 2. Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application.

different, hence a general estimate of error is untrustworthy and has been omitted from the diagrams. The main interaction effects revealed in the data are as follows :

Interaction of phosphorus and potassium $(P \times K)$. In series M interaction is almost absent at sample 1, but by sample 3 it becomes apparent; while not very large, it is nevertheless maintained to harvest. The interaction is positive, i.e. application at the higher level of both elements together results in greater increase than the sum of the increases due to individual applications of the two elements at the higher level to different plants. In series C interaction is of the same type as in M; it occurs here at sample 1 and is maximal from sample 2–3; thereafter it declines, although at harvest it is still quite as pronounced as in M series.

Interaction of phosphorus and rubidium $(P \times R)$. In M series interaction is very slight in samples 1 and 2; it becomes important by sample 3 and is very pronounced at harvest. It arises from the fact that after sample 2 the mean response to both elements presented together is great, but to either individually is slight; while on the average rubidium application alone is of little value, and phosphorus alone definitely detrimental, when given together they more

than double the final yield. In series C the course of this interaction is similar; it develops even later, not being very noticeable at sample 3, although by harvest it is similar in magnitude to that in series M. Here at this time the mean response to either element alone is negative; to both simultaneously,
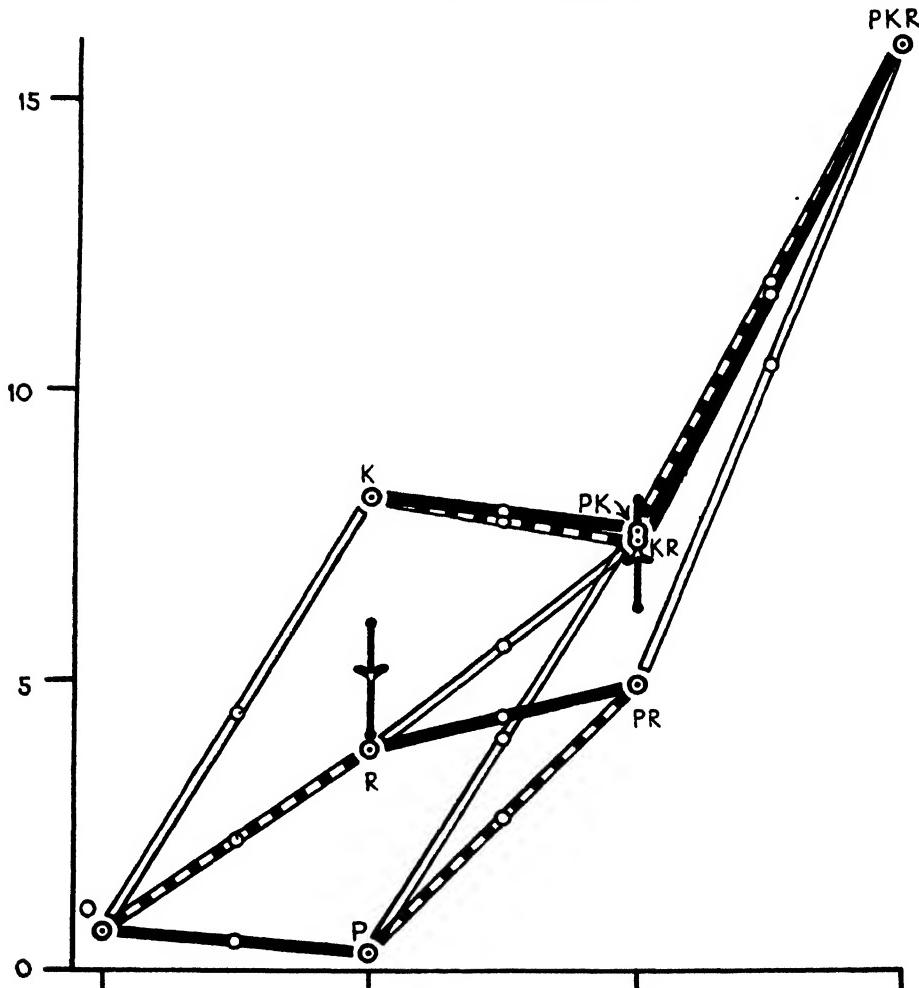
FIG. 3. Interaction diagram for total dry weight (gm. per pot) of series M, sample 3. Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application.

large and positive. This appears to be much the most important of the three first-order effects.

Interaction of potassium and rubidium $(K \times R)$. A fairly consistent small interaction occurs in M series of the opposite type to that of the previous two interactions, i.e. the absolute increase of yield due to rubidium is not so great at the higher potassium level as at the lower, and vice versa. In series C during

the sampling period interaction occurs similar in kind and magnitude to that in M, but by harvest it has reversed and become positive.

Interaction of phosphorus, potassium, and rubidium ($P \times K \times R$). Second-
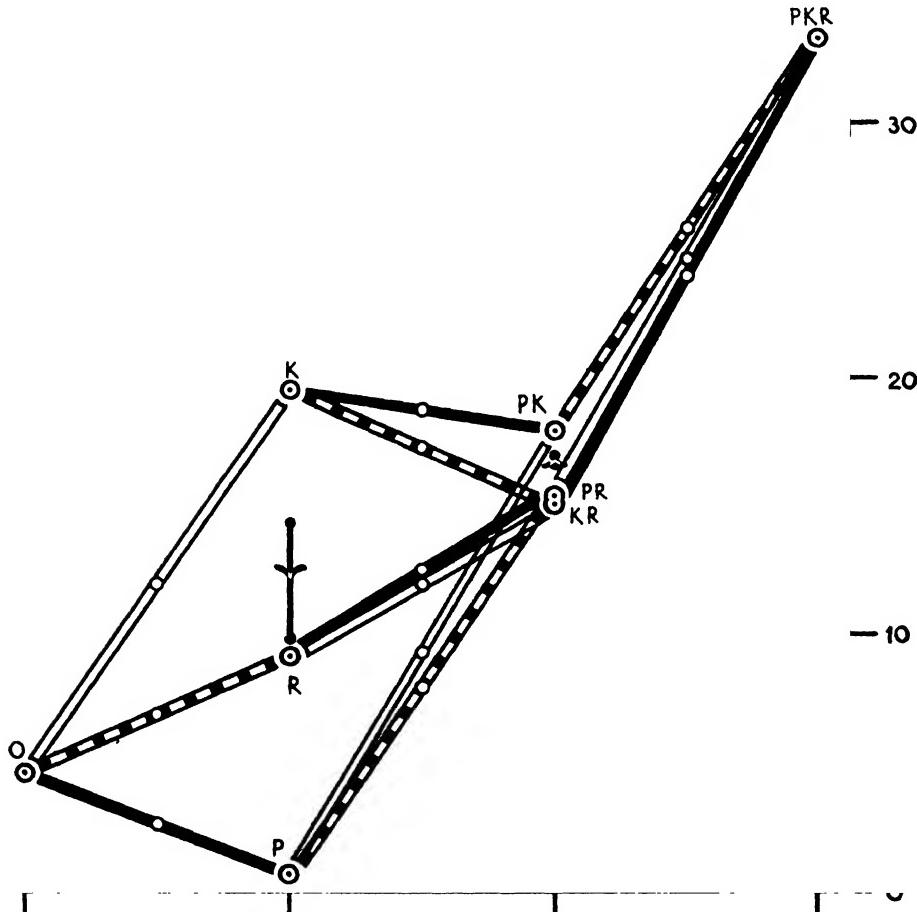
FIG. 4. Interaction diagram for total dry weight (gm. per pot) of **series M, final harvest**. Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application.

order interaction is almost absent in series M during the first two samples, while at the third and again at harvest it is considerable. Its introduction is due mainly to the rapid growth of treatment M : PKR relative to others between samples 2 and 3; so that while at the low phosphorus level there is little change in the negative interaction $K \times R$, at the high level the interaction changes rapidly at this time and reverses its sign. In series C the interaction

at both phosphorus levels is at first negative, being greater at the higher level, particularly at sample 2. By harvest interactions at both phosphorus levels have become positive, that at the high level being considerable. Thus the
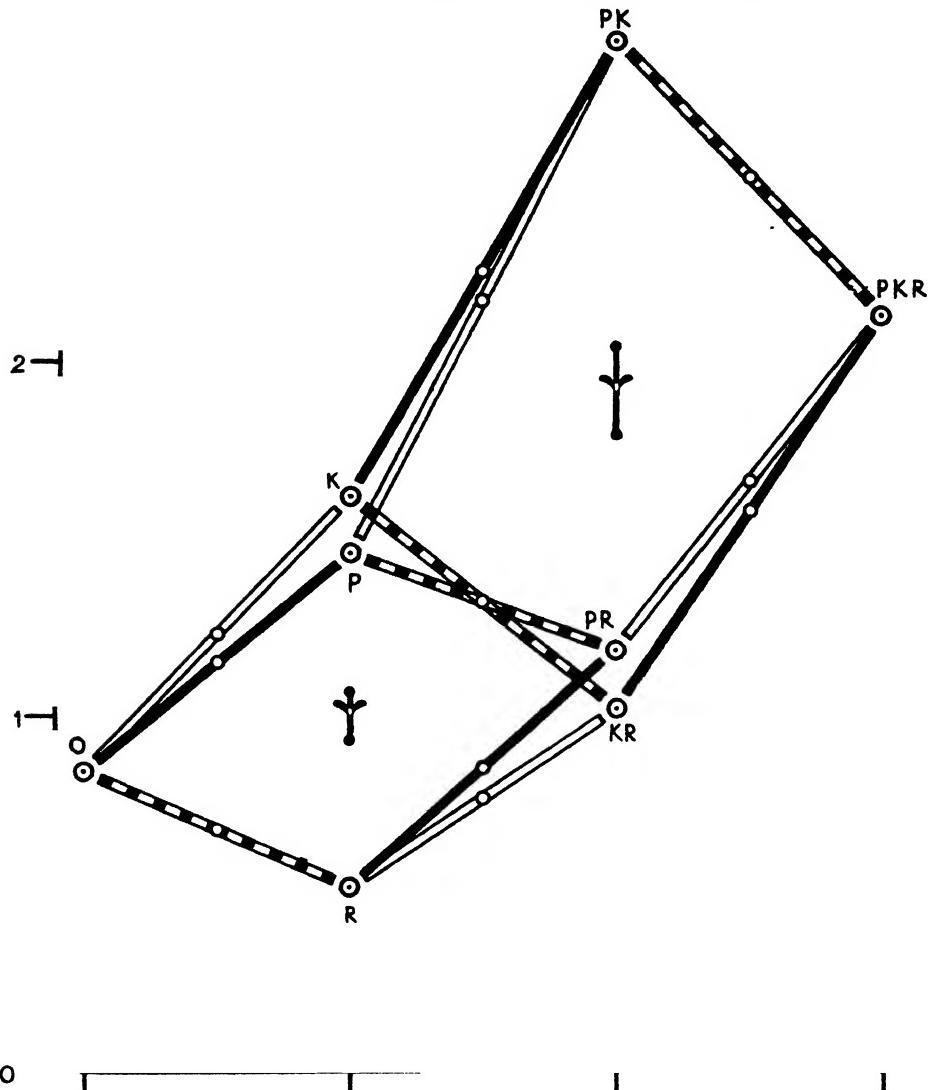
FIG. 5. Interaction diagram for total dry weight (gm. per pot) of series C, sample 1. Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application.

time changes of the second-order interactions of both series are in general similar; in series M the effect increases from zero to a high positive value, while in series C it increases from a moderate negative to a moderate positive value.

As to effects of the individual elements it may be pointed out that in series M phosphorus in the early stages is always detrimental or without effect; it finally results in a large increase of yield *provided that rubidium is present.* In
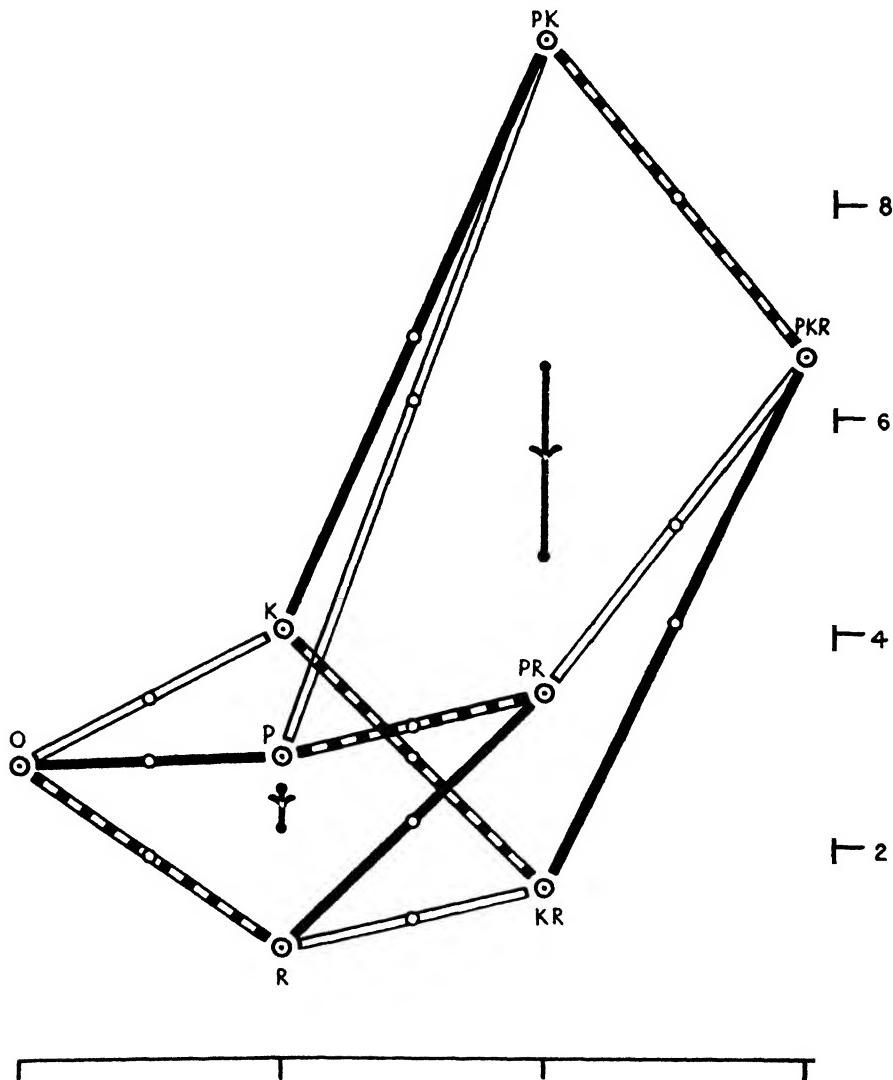
FIG. 6. Interaction diagram for total dry weight (gm. per pot) of series C, **sample 2.** Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application.

series C phosphorus everywhere increases yield greatly at sample 1; by sample 2 it becomes ineffective in the absence of either high potassium or rubidium (C : P); while under the same conditions by harvest it is highly detrimental. Accompanying potassium (C : PK) it is beneficial to a later stage,

but at harvest results in no greater yield than that due to potassium alone
(C : K). On the other hand, *if given with rubidium phosphorus addition is
highly beneficial throughout the life-history.*
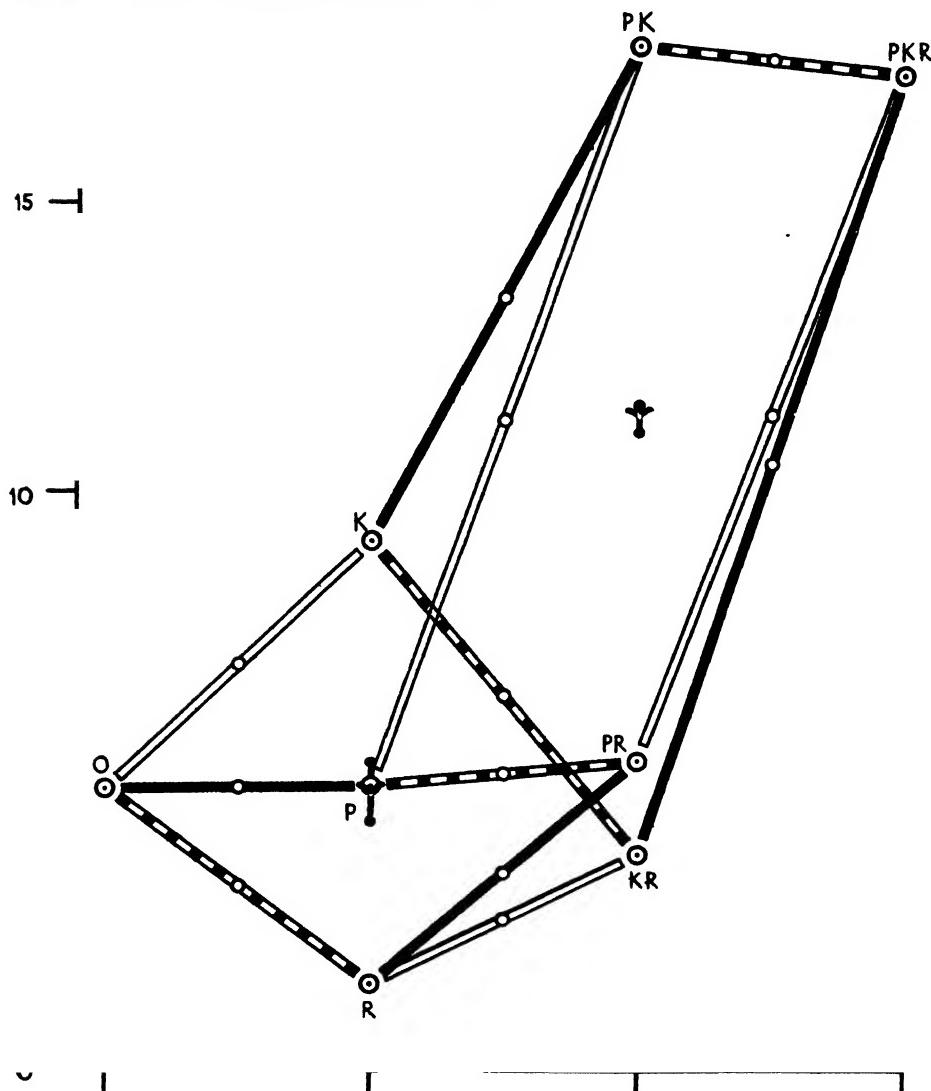
FIG. 7. Interaction diagram for total dry weight (gm. per pot) of series C, sample 3.
Black lines represent a difference of phosphorus level, white lines one of potassium level, and
pied lines one of rubidium application.

Increased potassium is everywhere beneficial, though in series M its effect
is considerably diminished by the presence of rubidium unless high phos-
phorus is also present. In series C a greater response to potassium is obtained
at the high phosphorus level than at the low throughout the main growth

period; by harvest this difference associated with phosphorus level largely disappears.

Effects of rubidium are considerable in both series. It eliminates the early
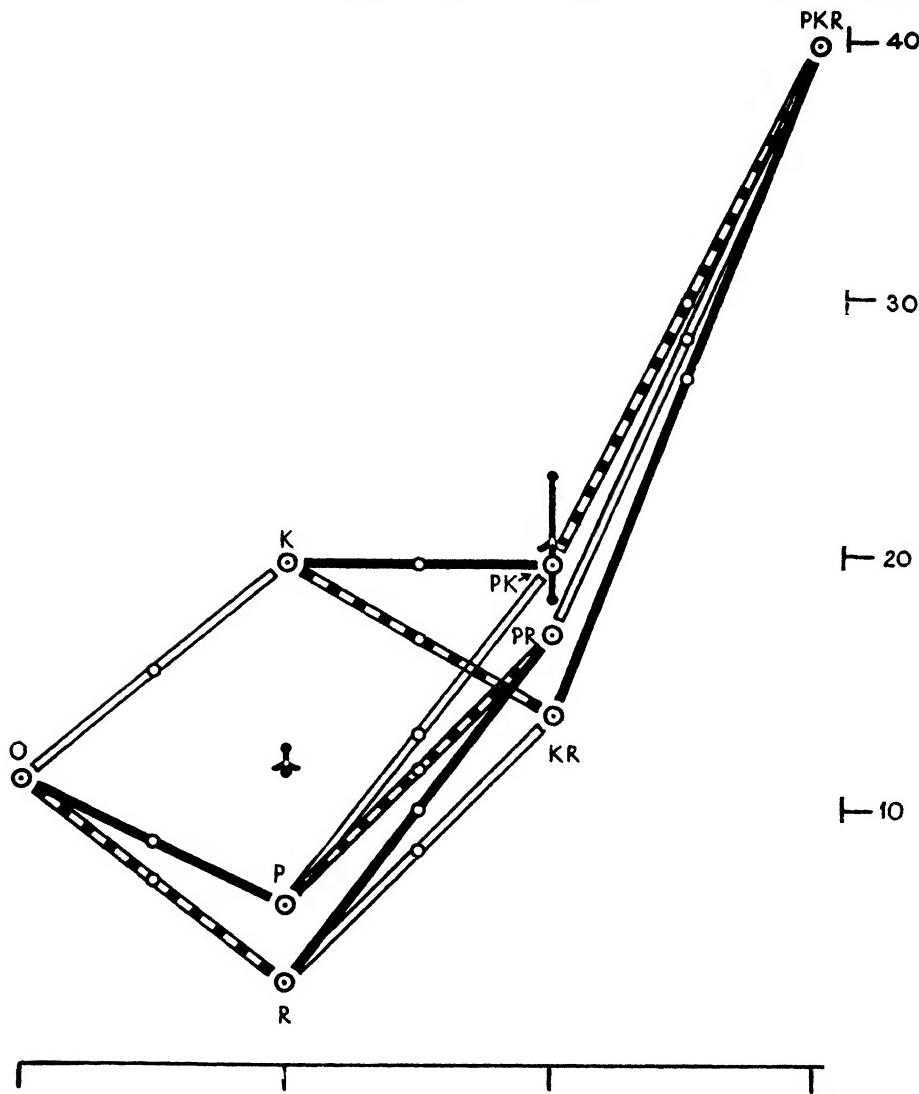
FIG. 8. Interaction diagram for total dry weight (gm. per pot) of **series C, final harvest**. Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application.

injury that destroys many plants of treatments M : O and M : P. In this series it is slightly detrimental when given at the high potassium and low phosphorus levels (comparison between M : K and M : KR); but at the high levels of both elements a greatly beneficial effect following rubidium applica-

tion becomes apparent after sample 2 (comparison between M : PK and M : PKR). In series C at sample 1 rubidium everywhere reduces yield, and remains equally deleterious to harvest at the lower phosphorus level. On the

TABLE IV

Mean Values of Total Dry Weight (gm. per pot)

Treatment.			Sample 1.	Sample 2.	Sample 3.	Harvest.
M : O	.	.	0·40	0·54	0·66	4·66
K	.	.	1·05	3·80	8·17	19·51
R	.	.	0·85	1·94	3·80	9·21
KR	.	.	1·06	3·05	7·44	15·22
M : P	.	.	0·19	0·22	0·30	0·66*
PK	.	.	1·02	4·38	7·55	17·98
PR	.	.	0·70	1·71	4·92	15·30
PKR	.	.	1·06	3·84	15·89	33·34
C : O	.	.	0·84	2·73	4·89	11·21
K	.	.	1·62	4·03	9·17	19·61
R	.	.	0·52	1·06	1·50	3·26
KR	.	.	1·02	1·61	3·74	13·66
C : P	.	.	1·46	2·83	4·94	6·33
PK	.	.	2·90	9·54	17·66	19·58
PR	.	.	1·18	3·42	5·33	16·84
PKR	.	.	2·12	6·57	17·13	39·87
X : P	.	.	0·71	1·65	3·04	8·74
PK	.	.	2·69	9·97	15·65	16·84
PR	.	.	1·33	3·31	7·64	20·36
PKR	.	.	1·91	8·16	19·80	40·38

other hand, in the two treatments in which rubidium and high phosphorus are both supplied detrimental effects have disappeared by sample 3 and at harvest large increases in yield ascribable to rubidium are found; these increases are as great as those within the M series.

The four treatments at the high phosphorus level from the calcium–ammonium (X) series give main factor and interaction effects for potassium and rubidium intermediate between those described for the M and C solutions; the sequence in time too is similar, hence they need not be further described.

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* This figure is too low. The treatment resulted in much premature death and great variability; during the fortnightly samples pots with no surviving plants were rejected, consequently at the final harvest the five pots contained only one living plant. The figure given is the weight of this small plant. In the 1938 experiments eight similarly treated pots had surviving plants at harvest; by including these the mean harvest weight becomes 4·91 gm. This figure, however, exaggerates the yield, since it ignores the fact that many pots carry no living plants to harvest.

C. *Phosphorus Content and Phosphorus Uptake*

In Table V are presented the results of the phosphorus analyses. The main features will be very briefly indicated. With the exception of treatments M : O and C : O the phosphorus content at the lower level of application at

TABLE V

Phosphorus Content (% dry-wt.) and Phosphorus Uptake

Treatment.	Sample 1	Sample 3					
	Leaf.	Leaf.	Stem.	Root.	Dead matter.	Whole plant.	Total uptake. (mgm. P_2O_5 per pot.)
M : O . .	1·06	0·99	1·62	0·84	1·02	1·01	7
K . .	0·73	0·29	0·38	0·38	0·19	0·33	27
R . .	0·51	0·30	0·38	0·21	0·17	0·29	11
KR . .	0·56	0·36	0·45	0·41	0·21	0·40	30
M : P . .	2·06	1·73	2·30	1·15	2·27	1·69	5
PK . .	1·96	1·70	2·01	1·92	1·52	1·79	135
PR . .	1·55	1·17	1·38	0·89	1·03	1·16	57
PKR . .	1·85	1·83	1·70	1·64	1·46	1·72	274
C : O . .	0·33	0·45	0·53	0·37	0·23	0·40	20
K . .	0·41	0·32	0·35	0·28	0·17	0·30	27
R . .	0·28	0·31	0·30	0·21	0·09	0·27	4
KR . .	0·31	0·32	0·36	0·25	0·10	0·29	11
C : P . .	1·10	1·14	1·55	1·90	1·39	1·49	74
PK . .	0·94	0·85	0·93	1·00	0·60	0·86	152
PR . .	0·65	0·44	0·42	0·41	0·14	0·41	22
PKR . .	0·73	0·56	0·58	0·55	0·25	0·54	93
X : P . .	1·55	1·22	1·76	1·60	1·38	1·45	44
PK . .	1·01	1·07	1·07	1·02	0·75	0·98	153
PR . .	0·67	0·51	0·57	0·43	0·18	0·50	38
PKR . .	0·95	0·73	0·67	0·55	0·21	0·63	125

the time of sample 3 is similar in corresponding treatments from the M and C series; the whole plant content in seven of the treatments here is not far removed from 0·3 per cent. and this presumably represents the internal level at this stage when phosphorus is limiting growth. The total amount taken up on the contrary is very different among these treatments; thus C : K has nearly seven times as much as C : R, though the contents are almost the same, i.e. the growth made is proportional to the phosphorus uptake. At the high phosphorus level the content in series M is always greater than in C, and except for treatments M : P and C : P this difference is considerable. With high phosphorus in the absence of rubidium the total uptake at sample 3 is greater in series C than in M, but in its presence that in M is considerably

greater than that in C. In C series rubidium always reduces the total phosphorus uptake; at high phosphorus levels this is reflected in much reduced contents, and at low phosphorus levels in reduced growth. In M series, on the contrary, rubidium increases total uptake. Owing to its simultaneous effects on growth, at the low potassium level rubidium decreases phosphorus content, while at the high level it has little effect.

The four treatments from the X series yield results intermediate between those of corresponding treatments from the M and C series, and more similar to C than to M.

DISCUSSION

In reviewing the data it appears difficult to escape the conclusion that the effects of rubidium are closely related to phosphorus nutrition. The experiments of Shih (1934) show that much less potassium is taken up from high calcium solutions than from those of high sodium, and also that uptake of phosphorus is much reduced by high calcium levels, particularly in solutions with low potassium. With high calcium supply the internal content of phosphorus varies only slightly with potassium level, while with high sodium and low calcium supply the phosphorus content markedly increases with decreasing potassium level. In these respects the effects of the present M and C solutions are similar to those of the high sodium and high calcium types of Shih, and the C series in general has considerably lower internal phosphorus content than the M. Treatments at the low phosphorus level naturally show much lower contents than those at the high.

It appears likely, and was indeed postulated by Shih, that excessive absorption of phosphorus leading to high internal contents is detrimental to growth. Such an excess is likely to be the primary cause of the greater toxicity and smaller plants throughout life in treatment M : P than in M : O. In the C series the treatment C : P was considerably better than C : O at sample 1; at sample 2 there was no difference in yield, while finally C : O yielded almost double the weight of C : P. In this series the rate of absorption of phosphorus is likely to be considerably lower than in M,[1] and indeed the plants from treatment C : O develop quite marked symptoms of phosphorus deficiency in the early stages. As the phosphorus is gradually absorbed steady growth is maintained, though the deficiency symptoms remain. On the other hand, treatment C : P absorbs phosphorus more rapidly in the early stages, phosphorus-deficient symptoms do not appear, and growth is rapid. Eventually, however, excess phosphorus accumulates and growth slows down so that in yield they are finally overtaken by C : O.

The addition of potassium alone to treatment M : O results in a large increase of yield, while the addition of both potassium and phosphorus

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[1] At sample 1 the total dry weight of C : O is twice that of M : O, while the phosphorus content of the leaf is less than one-third that of M : O, i.e. the total phosphorus taken up is likely to be considerably lower in C : O in spite of the growth rate being much greater.

(M : PK) leads to no greater yield than does potassium alone, and in other respects M : K is superior to and more normal than M : PK in later stages. On the contrary in C series the addition of both potassium and phosphorus (C : PK) results throughout the sampling period in a much greater increase than that given by potassium alone, as might be expected from the slow rate of entry of phosphate in this series and the fact that the C : K treatment displays marked symptoms of phosphorus deficiency. The difference during the sampling period between the M and C series in response to these two treatments is striking; at samples 2 and 3 there is little difference in weight between the K treatments of the two series, while the KP treatment of C has more than double the weight of that of M. But the early promise of C : PK is not maintained, and between sample 3 and harvest almost no further increase in weight occurs. This may very probably be explained as a delayed excess phosphorus effect.

In further support of the contention that excess phosphorus has considerable deleterious effects the phosphorus contents of the dead leaves may be cited (Table V): these are highest in those treatments presumed to be suffering from excess internal contents. A comparison between the living and dead leaf contents also indicates that, with the possible exception of treatment M : PKR, which will be considered later, these treatments provide the only plants of the experiment from whose leaves much of the phosphorus does not disappear at death.

The effects of rubidium addition may now be examined in the light of the above interpretations, making the simplest possible assumption as to the connexion between rubidium supply and phosphorus nutrition, i.e. that in the presence of rubidium, under otherwise similar conditions, absorption of phosphorus is reduced. In general, where the internal content of phosphorus is already excessive, resulting in deterioration to the plant, rubidium application should give improvement provided that any directly toxic effects of the element are less than the beneficial effects resulting from the prevention of excessive accumulation of phosphorus. Where, on the other hand, growth is already limited by phosphorus supply, rubidium addition should still further retard it and enhance any symptoms associated with phosphorus deficiency. It is apparent that many of the observed effects of rubidium may be accounted for in these terms.

The treatments which in the absence of rubidium are already phosphorus-limited are C : O, C : K, and probably M : K; M : O is presumably not so limited since it remains excessively dwarf, while its phosphorus content is high. C : O and C : K are clearly largely phosphorus-limited, and show the characteristic deficiency symptoms. The addition of rubidium to both is decidedly inimical to growth throughout the life-history: size is much reduced, tillering checked, net assimilation rate lowered, and all the usual colour and other symptoms of phosphorus deficiency are greatly enhanced. Phosphorus content in all four treatments is very low and approximately

constant, hence it appears that the growth made is conditioned almost entirely by the absolute amount of phosphorus taken up, and rubidium affects this in a striking manner. Both of these treatments which respond negatively to rubidium application respond at first positively and markedly to the raising of the external phosphorus concentration by ten times. The response of the C : P treatment to the much enhanced phosphorus supply is only temporary, and that of the C : PK scarcely extends beyond the sampling period, so that eventually treatment C : P gives considerably smaller plants than does C : O, and C : PK no larger than C : K. The conclusion appears inevitable that in the C series neither of the phosphorus levels used is well balanced physiologically with either of the potassium levels, and that for maximum efficiency both potassium levels would require a phosphorus level intermediate between those employed in the experiment. At the first sample both treatments C : P and C : PK respond negatively to rubidium, presumably because the rate of entry of phosphorus from the high calcium solution is slow even at the high phosphorus level; thus at sample 1 the leaf phosphorus contents are only half those of the corresponding treatments M : P and M : PK. Eventually, however, when they have suffered from the detrimental effects of excess phosphorus, both show an exceedingly high response to rubidium.

In consequence, therefore, in the high calcium series at sample 1 all the rubidium responses are negative, and all the phosphorus and potassium responses positive. With this solution the rate of entry of phosphorus in these early stages is as important a factor as supply of potassium, and any element such as rubidium which reduces the rate of entry inevitably slows down growth. By harvest both rubidium treatments at the low phosphorus level still occasion negative responses, but at the high phosphorus level rubidium now induces positive and large responses. In this series the magnitude of the interaction between phosphorus and rubidium is by harvest time very marked, since at the low potassium level application of either phosphorus or rubidium alone decreases yield very considerably, whereas the addition of both together just as effectively increases yield; an equally large and very similar interaction occurs at the high potassium level. The inference from the growth data is therefore that as regards final dry weight treatments C : O and C : K are partially limited by phosphorus concentration, treatments C : R and C : KR, owing to the rubidium, are more strictly limited by it, treatments C : P and C : PK suffer from excess phosphorus, while treatments C : PR and C : PKR are internally close to their optimum phosphorus levels and give very large yields for their respective potassium levels. The percentage phosphorus contents of the plants from these eight treatments at sample 3, together with the absolute contents, are fully in accord with these deductions.

In series C only the striking interactions between phosphorus and rubidium at harvest are outstanding, those between phosphorus and potassium on the one hand and between potassium and rubidium on the other being of a much lower order. This serves to throw into sharp contrast the effects of rubidium

and potassium, which appear to be largely additive and not closely related in nature. *At the low phosphorus level the effects on yield of these two elements are diametrically opposed*, and both effects are large; at the high phosphorus level the final effects of both are also large, but are in the same sense and of approximately equal magnitudes. Rubidium effects therefore are dependent very largely on phosphorus level, not only for their magnitude but even for their direction, while potassium effects are largely independent of phosphorus level over a wide range of the latter. With high potassium it is true that the internal content of phosphorus may be lower than with low potassium, but this is unlikely to be due appreciably to restriction of phosphorus uptake in the manner postulated for rubidium, and indeed the total uptake by the plant is considerably increased; the reduction in content is rather a direct consequence of the greater growth made. Increase of potassium supply leads in an unknown manner to more rapid growth and this in itself effectively prevents the excess and deleterious accumulation of phosphorus which may occur in its absence. Potassium minimizes phosphorus accumulation by more efficient usage and internal dilution; rubidium by retarding the rate of uptake. Thus at sample 3, treatment C : P has the high internal phosphorus content of 1·5 per cent.; potassium (C : PK) reduces this to 0·86 per cent. and rubidium (C : PR) much further, to 0·41 per cent. But whereas rubidium reduces the total uptake in C : P to below one-third, potassium more than doubles it; so that total uptake is seven times as great in C : PK as in C : PR. Addition of both elements (C : PKR) leads to intermediate results; hence the addition of rubidium to C : PK again restricts phosphorus entry, while the addition of potassium to C : PR not only increases total uptake, but even internal content somewhat (0·54 per cent.).

There still remains to consider behaviour within the M series. The interpretation of the results here must necessarily be more uncertain than with the C series, since there is the added complication of ammonium toxicity. While the general form of the interaction diagram in the two series is very different in early samples, at harvest there is close similarity.

In contrast with the C series, the addition of phosphorus to treatment M : O is deleterious from the earliest sample onwards, and this probably indicates that some of the toxicity appearing even in M : O is due to excess phosphorus. This conclusion is supported by a subsidiary experiment in which the phosphorus level of treatment M : O was reduced much lower (to one-eighty-first of the level here designated P), resulting in plants in which the toxic symptoms were absent; they were replaced by fairly typical phosphorus deficiency symptoms, while early growth was much increased. It appears then that a high internal content of phosphorus is a necessary preliminary to the production of these toxic effects. In spite of the low external level of phosphorus in treatment M : O the potassium level is yet lower and the plants remain very dwarf; there is no check on the rate of phosphorus absorption by any of the main constituents of the solution such as occurs in the C series,

and consequently the internal content is high. The addition of rubidium to each of these treatments (M : O and M : P) is immediately and greatly beneficial and the improvement is continued to harvest. Until the third sample there is no evidence of interaction between phosphorus and rubidium here, neither indeed is there at the higher potassium level, although by harvest considerable interaction is found at both levels. Interaction is then greater at the higher level and is exactly similar to those already described in the C series; it appears likely that at this time a similar explanation applies to both series at the higher potassium level.

At the low potassium level, while the general disposition of the four treatments at harvest is somewhat similar to those at the high level and also to those within the C series, yet here treatment M : R is relatively much higher, and in fact provides the only instance throughout the experiment of a beneficial effect of rubidium with low phosphorus supply. If the explanation is wholly of the same general kind as in C series we must assume that the plants of treatment M : O are suffering solely from excess internal phosphorus content and that the addition of rubidium reduces this, and in so doing eliminates the cause of the toxic action within the treatment. Phosphorus uptake must now be presumed to be below the optimum, since the addition of this element (M : PR) results finally in a further considerable increment in yield. Among these treatments, therefore, decreasing phosphorus content should be found in the order M : P, M : O, M : PR, M : R, the first two suffering considerably from a deleterious excess and the last limited by its reduced uptake. Now treatment M : R at sample 3 has as low a phosphorus content as any of the low phosphorus treatments from series C, hence the deduction that it is in fact phosphorus-limited is justified. Moreover, treatment M : O has a phosphorus content of the same order as C : P, which is clearly suffering from an excess, hence it is reasonable to suppose that M : O may be similarly affected. But as between treatments M : PR and M : O some other factor of as much importance as total phosphorus content is operative, since the former has actually a rather higher content than M : O. Evidence will now be presented that this other factor may be free ammonia content, and that the development of the toxic symptoms associated with M : P and M : O is dependent on the simultaneous presence of free ammonia and excess phosphorus within the plant.

Nitrogen fractionation of plants from certain treatments including M : P, M : PR, and one similar to M : PK but at a higher potassium level, at the early stage when the toxicity symptoms in M : P are developing, reveal only comparatively slight differences. Total nitrogen, protein, amide, and amino-nitrogen contents of fresh material are very similar within all series, amide being everywhere very high, but there is consistently a much higher content of free ammonia in the series which show the toxic symptoms than in those which do not. The accumulation of ammonia is not due to appreciably different rates of absorption of nitrogen, since the total nitrogen contents are almost identical; if therefore, as appears probable, the accumulation is one of the

primary causes of failure in these plants, the beneficial effects of both potassium and rubidium cannot be derived from reduced nitrogen absorption. Hence it is unlikely that the main effect of rubidium results from a *general* lowering of permeability to ions. Rather it would appear that potassium and rubidium are alike, and are probably unique among the elements, in that they enable the incoming ammonium to be transformed into innocuous substances as rapidly as it is absorbed. Whatever the effect, it is certain that lithium, sodium, and caesium cannot exert it, and the addition of these substances simply results in greater toxicity and more complete deterioration.

It is of course *a priori* possible that the accumulation of ammonia in the absence of potassium and rubidium, accompanying rapid deterioration, is solely due to the fact that these two elements alone when applied to the M : P treatment allow the plants to grow sufficiently rapidly to utilize the ammonium as quickly as it enters. In order to test this hypothesis plants were grown with the same ammonium solution but at a high potassium level and a very low phosphorus level. By these means early retardation of growth was induced, but toxicity symptoms with high mortality rates were conspicuously absent and nitrogen fractionation gave no evidence of internal accumulation of free ammonia. If, therefore, the toxic effects are largely due to the free ammonia content, growth rate is unlikely to be of primary importance in the matter and we are left with a specific effect of potassium, or rubidium. It is still possible, however, that the toxicity is primarily due to the deleterious effects of a large excess of phosphorus, and that the accumulation of ammonia is itself consequent on the high phosphorus content. Successful growth of such plants when given rubidium would then be due solely to the restriction of phosphorus entry.

It therefore appears established that a high internal phosphorus content is one of the essentials for the production of the toxic effects under discussion. Whether the simultaneous accumulation of ammonia is also essential is undecided, though it appears likely to be. Shih (1934) found similar high phosphorus contents in low potassium plants grown in a high sodium solution; here toxic symptoms also develop, but milder and quite distinct in character. Shih considered that for the development of the symptoms of his plants the simultaneous internal presence of excess phosphorus and excess sodium was requisite. It is likely then that the toxicity of M : O and M : P treatments is due to the simultaneous accumulations of both phosphorus and ammonia—possibly to the simple accumulation of ammonium phosphate within the plants.

In recent years similar disastrous effects of potassium deficiency in solutions containing ammonium salts have been described, in particular by Turtschin (1934), Schropp and Arenz (1939), and Wall (1940), and it is claimed that the observed toxicity is a direct consequence of the accumulation of free ammonia within the plant in the absence of potassium. Indeed, as has been mentioned, in the present experiments at the time of inception of the

adverse symptoms there is about a five-fold increase in ammonia content compared with the high potassium controls. Wall's data on the contrary show no increase in ammonia content of the leaves at the time of his first sample, though the stems do show a small rise. It appears unlikely, therefore, that the breakdown symptoms exhibited by his plants at this time can be accounted for solely in terms of ammonia accumulation, but since his estimations were made on oven-dried material they may be open to criticism (cf. Richards and Templeman, 1936).

Again, Wall (1939, 1940) believes that the main effect of potassium deficiency is to check protein synthesis at the stage of amino acids. Potassium is supposed to be directly concerned at this stage, a hypothesis with which the present author is not in agreement, and which in his view Wall's own data do not support. The resulting accumulation of amino acids is primarily responsible for the accumulation of ammonia and development of toxicity. Now protein synthesis can certainly be checked at this very point by deficiency of phosphorus, leading to as high or even higher concentrations of amino acids and amides than does potassium deficiency. If Wall's contention is correct, therefore, ammonia content should rise here also with the development of similar toxic symptoms, but as has been indicated this is very far indeed from being the case, whatever the level of potassium.

The total rubidium effect over the experiment, therefore, may possibly be threefold: (1) a general effect, always adverse, ascribable to a direct toxic action of the element itself; at the low rubidium level used this toxicity is not great and its effects may be considerably outweighed by other beneficial effects; (2) an effect, either beneficial or adverse, and related to conditions of phosphorus supply; it probably consists in a retardation of the rate of phosphorus entry; (3) a more doubtful special effect, sometimes highly beneficial, related to the removal of toxic ammonium. In the first two effects rubidium stands in rather sharp contrast with potassium, in the third it alone of all other alkali metals resembles potassium.

If these deductions are correct it follows that the total effect of potassium within the plant may be analysed into two parts, for one of which replacement by rubidium is possible, while for the other potassium is unique.

The remaining four treatments from the M series receiving potassium at the higher level are difficult to account for along the lines already put forward. The phosphorus contents fall into two groups, according to the level of supply. Treatments M : PK and M : PKR have almost identical contents, rubidium apparently exerting here only negligible restriction on phosphorus entry; similar considerations apply to treatments M : K and M : KR at the lower phosphorus level. Moreover, the contents in the former pair are as high as in M : P, which is suffering greatly from excess phosphorus. If phosphorus content is the sole determiner of the toxic effect, then the best measure of toxicity is not the total phosphorus but some fraction, possibly inorganic phosphate; or else plants grown in different nutrient solutions are able to

withstand different internal concentrations of the toxic substance, and with higher potassium greater internal concentrations of phosphorus are possible.

If, on the other hand, as seems likely, considerable poisoning develops only in the simultaneous presence of excess phosphorus and ammonia, then it must be assumed that the higher potassium concentration checks for some time the accumulation of free ammonia, but that after sample 2 the potassium of M : PK is no longer sufficient for this purpose and deterioration sets in relative to M : PKR. It is probably significant that at this time in the treatment M: PK, and also in M : K, deterioration begins very characteristically with a yellowing and dying of fairly young leaves, and not of the lowest and oldest leaves which remain green for some time longer. Presumably the early leaves outlive the younger ones owing to their higher potassium level. This hypothesis will account for the fact that M : PKR continues to grow rapidly long after M : PK, since rubidium is as effective as potassium in countering ammonia accumulation. If this interpretation bears any relation to the truth, it follows that treatments M : K and M : KR become phosphorus-limited by sample 3, although the delay in their early growth shown by a comparison with C : K at sample 1 cannot be due to any such limitation, again judging by the comparison between the phosphorus contents of their green leaves and those from the C series. Presumably this early delay is due to ammonia accumulation. Where phosphorus cannot also accumulate (M : K and M : KR) the setback is temporary, so that by sample 2 these treatments have almost caught up with C : K; but where, as in M : PK, high phosphorus content is associated with the ammonia, the potassium level is insufficient to counteract the effects of both these elements, and M : PK lags far behind C : PK through the whole sampling period and symptoms of disorganization appear much earlier. The initial delay in M : PKR relative to C : PKR is on the contrary largely eliminated by sample 3.

The most obvious objection to this interpretation is that at harvest the interactions of treatments K, PK, KR, and PKR from both series are closely similar as also indeed are the dry weights of corresponding treatments, and it is unlikely that such similar effects have been brought about by different causes. On the other hand, the similarity between the two series at harvest is evidently not dependent on close similarity of corresponding treatments through the growing period, and the interaction diagrams from the early samples are very different; hence the explanations of the total effects in the two series cannot be identical.

SUMMARY

1. Experiments are briefly described which demonstrate that while barley may grow successfully in a high potassium nutrient solution containing ammonium salts, only a minimum of calcium, and without sodium, it is in such circumstances much more sensitive to potassium deficiency than is usual with other types of nutrient solutions. With very low potassium levels growth

nearly ceases at the first or second leaf stage and a large proportion of the plants die. The addition of rubidium to the solution enables early growth to proceed almost normally. If the rubidium level is high, characteristic abnormal symptoms soon appear and may again be followed by premature death; but a range of rubidium concentrations exists over which the element increases total growth many times. Sodium, lithium, and caesium do not have this effect.

2. The effect on growth of rubidium depends in a complex manner on the calcium-ammonium status of the solution and the level of phosphorus supply. The main experiment described in the paper was set up to investigate more thoroughly this interaction. Three series of nutrient solutions were used: high ammonium with low calcium (M), high calcium without ammonium (C), and intermediate calcium and ammonium (X). These were combined with a high and low level of phosphorus, two levels of potassium both low, and absence and presence of rubidium. The nomenclature scheme and salt composition of the twenty solutions are given in Tables I and II. Three fortnightly samples were taken during the growing period together with a final harvest.

3. External symptoms accompanying excessive rubidium supply are described, together with the responses in type of growth produced by the various nutrients of the main experiment. With excessive rubidium the roots remain short and become thick, while early leaves are wide, dark green, waxy, and brittle with prominent mid-ribs and exaggerated twisting. Later leaves are a light grey-green and exceedingly short and narrow. At this stage the plants are short and bushy, with excessive numbers of thin tillers; many plants now die, and the survivors do not form ears.

4. The total dry weight data are presented in Table IV and as interaction diagrams in Figs. 1–8. Potassium leads everywhere to large increases in growth. In early stages, high phosphorus may be detrimental or without effect (series M), or beneficial (series C); it is always beneficial at harvest *provided rubidium is present*, but is usually harmful in its absence. Rubidium eliminates the early toxic action appearing in high ammonium–low potassium solutions. In all treatments receiving high phosphorus at either potassium level rubidium is *finally* greatly beneficial; though at low phosphorus level it may be detrimental, particularly in high calcium solutions. Treatments from the intermediate series (X) provide results intermediate between those of the series C and M.

5. The results of the phosphorus analyses of all plant fractions from sample 3 and of the leaves from sample 1 are presented in Table V. They indicate that at sample 3, where phosphorus is given at the low level, phosphorus content in general tends to a constant minimal value, though the total amount taken up varies widely among the treatments, i.e. growth is largely conditioned by phosphorus uptake. At the high phosphorus level series M has higher contents than corresponding treatments from series C. In the C series rubidium

always reduces total phosphorus uptake, but in M series increases it, though the percentage content may be reduced.

6. The complex effects of rubidium application on growth are examined from the standpoint that the total effect is at least twofold and probably three fold: (1) a general toxic effect specific to the element, responsible for the symptoms described in paragraph 3; (2) an effect dependent on a retardation of the rate of phosphorus entry, especially from high calcium solutions; and (3) a more doubtful effect related to the removal of toxic ammonium ion. In the first two effects rubidium stands in contrast to potassium, in the third it alone of the other alkali metals may resemble potassium. The total potassium effect within the plant therefore, may probably be analysed into two parts, for one of which (removal of toxic ammonium) replacement by rubidium is possible, while for the other potassium is unique.

7. On the assumption that excessive accumulation of phosphorus within the plant is injurious, the effect of rubidium on the uptake of that element may account for many of the results of the experiment. Reduction in phosphorus content following rubidium application to high calcium–high phosphorus treatments leads to improved growth and general condition. In treatments with low phosphorus supply, where growth is already restricted from this cause, rubidium addition is followed by further reduction in growth and the accentuation of external symptoms of phosphorus deficiency.

8. While lowering the phosphorus level of the high ammonium treatment at low potassium levels reduces the toxicity of the treatment, it is likely that the severe injury found under these conditions is partly due to the ammonium ion, i.e. to internal accumulation of both phosphorus and ammonium. Both these deleterious accumulations are countered by rubidium, hence the strikingly beneficial effect of this element here.

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Adaptation and Counter-Adaptation of the Breeding System in Primula

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With one Figure in the Text

THE NATURE OF BREEDING SYSTEMS

THE system of breeding or mating of the individuals composing a population is one of the main agents determining the storage and rate of release of heritable variation. Thus, inasmuch as the response to a given selective force is conditioned by the available variation, the breeding system plays a large part in controlling the rate and magnitude of selective changes.

Now the morphological and physiological characteristics of any individual are controlled by its genotype, and will be changed to a greater or less degree by alteration of the constituent genes. Such changes not only affect the gross fertility of the individual in question but, in altering its mating habits, will also be reflected in changes in the rate of outbreeding, where this term is used in its broadest sense to cover both outcrossing and choice of mate. The rate of outbreeding is of importance in that it determines the degree of hybridity of the offspring, upon which depends the release of variations, and hence the progress of response to selection in later generations.

Thus the genes which, by reason of their morphological and physiological effects on the individual, control the system of breeding of the population ultimately play an important role in the determination of the reaction of that population to selected stresses. In other words the breeding system is adaptive. Those genes which affect it will be subject to selection by virtue of their action in exposing other genes and gene combinations to, or sheltering them from, the rigours of selective action.

Two important consequences follow from this conclusion, viz. that any consideration of change in a population under the action of selection must include a suitable genetical analysis of the system of breeding of the population; and that this analysis may properly be based on those principles which have been developed from the consideration of other kinds of adaptation.

The suitability of any breeding system is determined by the amount of outbreeding or, speaking inversely, of inbreeding which it produces. We may then make a broad classification into the three types, essentially inbreeding systems, essentially outbreeding systems, and mixed and balanced systems. This classification is not precise without some measure of the rate of inbreeding or of total heterozygosity, and no such system has yet been sufficiently well developed. Present needs can, however, be met by a rough classification, since this introduces the necessary concept of direction of change, as being towards inbreeding or towards outbreeding.

Whatever the optimum rate of outbreeding or inbreeding, the adjustment of the breeding system to the production of this optimum involves control of the system. Furthermore the adjustment may be made with greater or less efficiency, and so the question of the degree of control of breeding systems is introduced. Here again precise classification would necessitate a measure of the control, and such a measure does not exist. As before, however, by recognizing that such a control exists to a greater or less degree one is enabled to recognize and specify the direction of change, and this is sufficient for present purposes.

Though it is generally possible and frequently easy to say that one breeding system is more highly controlled than another related type it is impossible to define an uncontrolled system. The implication is clearly not one of randomness, as the existence of obstacles in the environment would render random mating impossible even where the system was genetically of a suitable type. Furthermore the nature and distribution of the reproductive organs must always exert some influence on mating. The definition of uncontrolled systems is not, however, necessary for the discussion of selective changes. In geometric terms the analysis of directional changes requires only a line of reference and involves no assumptions as to the position of the origin.

Many methods of controlling breeding are to be observed in nature. They may be classified according to whether they require genetical homogeneity or genetical heterogeneity for their working. In the homogeneous class are such devices as protandry and protogyny which result in increased outbreeding and premature anthesis which gives rise to inbreeding. In the heterogeneous class are to be found sex separation, incompatibility, heterostyly, and other outbreeding mechanisms. We may note that controlled inbreeding does not require, and indeed is hindered by, genetical heterogeneity, whereas the genetically homogeneous system can never encourage outbreeding so well as a heterogeneous system. The latter type would seem to have an additional advantage in that changes in the proportions of the various genotypes would

lead to relatively rapid adjustment of the breeding system to alteration in the outbreeding optimum.

Heterogeneous systems also present special problems relating to their origin and maintenance. Where two allelomorphs exist at a given locus a very small relative advantage of one will result in its spreading until the other becomes rare in the population. Thus for both to be maintained, as in breeding systems of the type under consideration, special conditions must have been present to produce the heterogeneity, and must still operate to maintain it. Careful genetical analysis coupled with comparison of the systems of related forms can often do much to elucidate the progress of these events. Sex separation has been widely discussed from this point of view (see Darlington, 1939) but little attention has been given to other mechanisms.

Sufficient evidence of this kind is now available to justify a preliminary discussion of heterostyly, which though not nearly so widespread as sex, is nevertheless an outbreeding mechanism of some importance in plants.

HETEROSTYLY AND ILLEGITIMACY

Heterostyly is commonly described as a morphological means of encouraging outbreeding. This is, however, only a part and probably the less important part of the story, for as Charles Darwin realized in 1877 heterostyly is always associated with a physiological outbreeding mechanism known as illegitimacy. In a distylic type, such as various Primula species, not only are pin × pin and thrum × thrum pollinations less likely to occur for morphological reasons than pin × thrum and its reciprocal, but even if they do happen, the pollen fails to achieve fertilization. In tristylar plants like *Lythrum Salicaria* the situation is even more remarkable, as pollen from anthers of one level differs in behaviour from that produced by anthers at the other level of the same flower. Thus pollen from the long stamens of a short-styled plant will achieve fertilization when placed on a long style. Pollen from the mid stamens of the same flower would fail on a long style. Though the actual lengths of the stamens on any plant are determined by the genotype, the behaviour of the pollen is directly controlled by stamen length, its relation to the genotype being more complex.

It would thus appear that the real significance of the morphological differences shown by the pistils and stamens lies in the physiological differences which follow from them. The alternative view is that they are distinct outbreeding mechanisms which have developed side by side to give mutual reinforcement, neither being as satisfactory alone. This supposition is contrary to all the evidence. In the first place heterostyly is always accompanied by illegitimacy and, with the possible exception of Riley's (1936) Capsella case, illegitimacy by heterostyly. Neither is ever found to be accompanied by any other outbreeding mechanism. If they can exist independently it would be remarkable indeed never to find them either alone or with some third mechanism. They must be developmentally connected. The morphological

differences are presumably selected on the basis of the physiological differences which they produce.

Certain other considerations must, however, be noted. The monomorphic ancestors of heterostyled plants cannot have shown any illegitimacy reaction even though their floral morphology was such as to suggest, by comparison with their descendants, that they would. Thus the association between illegitimacy and heterostyly is capable of being built up and hence modified by genic selection. That this can occur has been shown by Ernst (1933), who found some Primula plants in which the association was actually reversed.

The genus Primula is well suited to an analysis of heterostylism and illegitimacy. Various modifications of the joint mechanism, notably that towards homostyly, are known, and in *Primula sinensis* the illegitimacy reaction is not so strongly developed as to preclude all possibility of genetical investigation of the mechanism. The wild form of *Primula sinensis* is not known, but this does not constitute any difficulty as it is clear that its heterostyly, like that of other species, has been developed in nature, and hence the conclusions drawn from the study of this plant are of general applicability.

The Genetics of Heterostyly in *Primula sinensis*

The data on the inheritance of heterostyly up to 1933 have been summarized by de Winton and Haldane. Further results have been obtained since that date and are given, together with the earlier figures, in Table I. This table includes segregations from three of the four possible types of mating, viz. thrum by pin (**Ss × ss**), pin by thrum (**ss × Ss**), and thrum by thrum (**Ss × Ss**). The fourth type, pin × pin, has given only pin progeny in extensive trials.

Table I[1]

Mating.	No. of families.	Progeny. S.	s.	χ^2.	P.
Ss × ss . .	230	5477	5546	0·432	0·5
ss × Ss . .	118	4309	4114	4·514	0·04–0·03
Ss × Ss . .	111	7502	2703	12·035	0·001–0·0001

The figures given in the last column are the χ^2s calculated for deviations from the expected 1 : 1 in the case of the two backcrosses and from 3 : 1 for the F_2 data. The deviation in the female backcross (**Ss × ss**) is clearly not significant, while that for the male backcross has a probability of about 3 per cent. and so is a borderline case. The F_2 segregation, however, is highly discrepant, with a probability of less than 0·1 per cent.

Such apparently significant departures from expectation could be obtained by the summation of heterogeneous data. Tests were undertaken to decide this question. In each type of cross χ^2 was calculated for each family separately,

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[1] This table includes the corresponding figures given by de Winton and Haldane.

and summed. The difference between this sum and the χ^2 calculated from the total segregation is itself a χ^2 testing heterogeneity and corresponding to a number of degrees of freedom one less than the number of families (see Mather, 1938, section 6). The χ^2 analyses obtained in this way are given in Table II.

TABLE II

			χ^2.	$d.f.$	$P.$
Ss × ss	.	. Deviation . .	0·432	1	0·7–0·5
		Heterogeneity. .	237·469	229	0·7–0·5
		Total . .	237·901	230	
ss × Ss	.	. Deviation . .	4·514	1	0·03
		Heterogeneity. .	132·053	117	0·40–0·30
		Total . .	136·567	118	
Ss × Ss	.	. Deviation . .	12·035	1	< 0·001
		Heterogeneity. .	113·182	110	0·9–0·8
		Total . .	125·217	111	

It will be seen that there is no sign of heterogeneity in any of the three types of mating. The significant deviation of the F_2 total must be explained in some other way.

Thus it appears that the F_2 frequencies are being influenced by something other than normal mendelian segregation of allelomorphs. Furthermore this effect is apparently peculiar to F_2 families, inasmuch as the two backcrosses show no such serious departures from expectation. This may be tested more rigorously in the following way.

If the F_2 shows no departure from expectation other than that which may also be present in the backcrosses, the proportion of **ss** plants in the F_2 will be the product of the proportions found in the two kinds of backcross. Whether this is so may be determined by the method developed, in a different connexion, by Mather (1940). This consists of calculating a χ^2 for one degree of freedom from the formula

$$\chi^2_{[1]} = \frac{(n_3 a_{12} a_{22} - a_{32} n_1 n_2)^2 n_1 n_2}{a_{12} a_{22} n_3 [n_1 n_2 (n_1 n_2 - a_{12} a_{22}) + n_3 n_1 a_{12} a_{21} + n_3 n_2 a_{22} a_{11}]} \qquad 1$$

where a_{11} &c., and n_1 &c. are the numbers of plants observed in the various categories according to the following scheme:

	Mating		
Progeny class	**Ss × ss**	**ss × Ss**	**Ss × Ss**
S . .	a_{11}	a_{21}	a_{31}
s . .	a_{12}	a_{22}	a_{32}
Total .	n_1	n_2	n_3

[1] $\chi^2_{[1]}$ means χ^2 for one degree of freedom.

Using this formula we find:

$$\chi^2_{[1]} = 11\cdot773 \text{ and } P = \, < 0\cdot001$$

So there is no doubt that the discrepancy is peculiar to the F_2 data.

Two obvious possibilities exist. These are (A) that the two kinds of pollen, **S** and **s**, produced by thrum plants have different growth ratios on thrum (**Ss**) styles, and (B) that thrum homozygotes (**SS**) have a lower viability than either thrum heterozygotes (**Ss**) or pin (**ss**). These latter classes are shown to have the same viability by the results from the female backcross (**Ss** × **ss**).

A decision may be made between the two possibilities by testing thrums from F_2 families for homozygosity. If the disturbance is due to differential pollen growth the F_2 family should contain the three genotypic classes in the proportions:

SS	**Ss**	**ss**
$\dfrac{x}{2}$	$\dfrac{1}{2}$	$\dfrac{1-x}{2}$

where x and $1-x$ are the frequencies with which **S** and **s** pollen achieve fertilization respectively. Hence the phenotypic segregation in F_2 will be $\mathbf{S}\dfrac{1+x}{2}$, $\mathbf{s}\dfrac{1-x}{2}$, which reduces to $3:1$ when $x = \frac{1}{2}$. The thrum component of the F_2 should itself be composed of $\dfrac{x}{1+x}$ homozygotes and $\dfrac{1}{1+x}$ heterozygotes. This is clearly $2:1$ when $x = \frac{1}{2}$.

Where, however, poor viability of the homozygous thrums is the cause of the disturbance different results are expected. Putting the viability of the thrums as v, the three F_2 classes are expected in the proportions:

SS	**Ss**	**ss**
v	2	1
$\dfrac{}{3+v}$	$\dfrac{}{3+v}$	$\dfrac{}{3+v}$

Hence an F_2 will show a segregation of $\dfrac{2+v}{3+v}$ **S** and $\dfrac{1}{3+v}$ **s** and the thrums will contain $\dfrac{v}{2+v}$ homozygotes and $\dfrac{2}{2+v}$ heterozygotes.

Thrum plants from three F_2 families were tested for homozygosity by crossing them as females with pin males. Sufficient plants were grown in each of the resulting families for the error of classification, due to failure of appearance of a pin in the progeny of a heterozygous thrum, to be negligible. The minimum number of progeny used was 9, and in the vast majority of cases this total was well exceeded.[1]

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[1] With nine plants the chance of misclassification due to failure of appearance of a pin in the progeny of a heterozygote is $(\frac{1}{2})^9$, i.e. $0\cdot001954$ or about 1 in 500.

The segregation for pin and thrum observed in these F_2s is shown in Table III together with the results of the testing programmes.

TABLE III

Family.	F_2 segregation.		Total.	Result of testing thrums.		Total.
	S	**s**		**SS**	**Ss**	
363/32 . .	113	46	159	30	76	106
4/39 . .	143	55	198	28	72	100
9/39 . .	79	25	104	8	31	39
Total . .	335	126	461	66	179	245

Using Brandt and Snedecor's method of calculation (Mather, 1938, section 7) χ^2 for heterogeneity between families is found to be 0·7724 for the F_2 segregation and 0·9755 for the results of testing the thrums. Each of these χ^2 values corresponds to two degrees of freedom and each fails to reach significance. The three families may thus be pooled for all further calculations.

First consider the possibility of pollen competition. The numbers observed are compared with the expectations following from this view (hypothesis A) in Table IV. The appropriate test of agreement with this hypothesis is that described by Mather (1938, section 19). It consists of finding the best fitting value of x from the pooled observations and testing the homogeneity with respect to x of the two sets of results. Heterogeneity indicates disagreement with hypothesis.

TABLE IV

	F_2 segregations.		Results of testing thrums.	
	S	**s**	**SS**	**Ss**
Observed	335	126	66	179
Expected on hypothesis $\begin{cases} A \\ B \end{cases}$	$\frac{1}{2}(1+x)$ $\frac{2+v}{3+v}$	$\frac{1}{2}(1-x)$ $\frac{1}{3+v}$	$\frac{x}{1+x}$ $\frac{v}{2+v}$	$\frac{1}{1+x}$ $\frac{2}{2+v}$

The first step is to estimate x. The joint log. likelihood is

$$L = 335 \log\left(\frac{1+x}{2}\right) + 126 \log\left(\frac{1-x}{2}\right) + 66 \log\left(\frac{x}{1+x}\right) + 179 \log\left(\frac{1}{1+x}\right)$$
$$= 90 \log(1+x) + 126 \log(1-x) + 66 \log x - 461 \log 2.$$

Then the best fitting value of x is given by the appropriate root of the maximum likelihood equation

$$\frac{dL}{dx} = \frac{90}{1+x} - \frac{126}{1-x} + \frac{66}{x} = 0.$$

Simplification leads to the quadratic

$$282x^2 + 36x - 66 = 0,$$

and $x = 0·424142$.

Substituting for x in the formulae of Table IV the following expectations are arrived at:

TABLE V

| | F$_2$ segregation. | | Thrum tests. | |
	S	s	SS	Ss
Observed (a) . .	335	126	66	179
Expected (mn) . .	328·265	132·735	72·967	172·033

The $\chi^2_{[1]} \left[= S\left(\dfrac{a^2}{mn}\right) - n \right] = 2 \cdot 661$. This has a probability of 10 per cent., which though not significant is suspiciously low.

We may next take the case of reduced viability of thrum homozygotes (hypothesis B). The expectations are set out in Table IV, from which is constructed the following log. likelihood expression for the purpose of estimating v:

$$L = 335 \log\left(\frac{2+v}{3+v}\right) + 126 \log\left(\frac{1}{3+v}\right) + 66 \log\left(\frac{v}{2+v}\right) + 179 \log\left(\frac{2}{2+v}\right),$$

which reduces to

$$L = 90 \log(2+v) - 461 \log(3+v) + 66 \log v.$$

Differentiation with respect to v gives as the equation of estimation

$$\frac{dL}{dv} = \frac{90}{2+v} - \frac{461}{3+v} + \frac{66}{v} = 0.$$

The quadratic obtained on simplifying is

$$305v^2 + 322v - 396 = 0,$$

and $$v = 0 \cdot 727920.$$

From this value of v the expectations for the various class are found to be:

TABLE VI

| | F$_2$ segregation. | | Thrum tests. | |
	S	s	SS	Ss
Observed . .	335	126	66	179
Expected . .	337·339	123·661	65·376	179·624

and $\chi^2_{[1]} = 0 \cdot 068$ which, with a probability of between 0·79 and 0·80, indicates excellent agreement with hypothesis.

These tests may be repeated using the whole of the F$_2$ data from Table I in place of the three families of Table III. The test families are, of course, the same as before. The following results are then obtained:

Hypothesis A (pollen growth) $x = 0 \cdot 468277$, $\chi^2_{[1]} = 2 \cdot 821$, $P = 0 \cdot 09$
Hypothesis B (reduced viability) $v = 0 \cdot 769226$, $\chi^2_{[1]} = 0 \cdot 096$, $P = 0 \cdot 76$.

These fully confirm the results of the early calculations.

Thus though the hypothesis of differential pollen growth is not unambigu-

ously rejected, its probability is low. The hypothesis of reduced viability of SS plants is, however, in much better agreement with the data and must be taken as the better explanation of the shortage of thrum plants in F_2.

Two results are then seen to emerge from these experiments: (1) thrum is dominant to pin, (ii) thrum homozygotes are at a disadvantage as compared with thrum heterozygotes and pins, the last two classes showing indistinguishable viability.

Before turning to the meaning of these results it is necessary first to mention what is known of the genetical modification of the manifestation of the pin–thrum difference. Two genes, first recognized by other effects, are known to affect the relative heights of anthers and stigma. One, the gene for Primrose Queen eye (**a**) shortens the style; the other, fertile double (**m**) raises the level of the anthers (de Winton and Haldane, 1933). Beale (1939) has shown that the illegitimacy reaction of the pollen and styles is similarly affected by these genes. Primrose Queen pin style behaves in mixed pollinations like an intermediate between pin and thrum, as does the fertile double pin pollen. This clearly has two implications. In the first place it shows that genes at other loci can modify the cross-breeding mechanism which is controlled by S, s, and secondly the modification is effected in both the morphological and physiological features of the mechanism. It may be added that this latter point supports the view developed in section 2, that the two mechanisms are not now separate in action, but that one determines the other.

THE DEVELOPMENT OF HETEROSTYLY

The key to the problem of the development of heterostyly in Primula is provided by the fact that although thrum is dominant to pin, the thrum homozygote is less viable than homozygous pin. Now Fisher (1931) has advanced reasons for believing that dominance is a property subject to selective control and that the heterozygote is selected towards resemblance of the more advantageous homozygote. So much is generally accepted, although the details of how selection acts to produce this result are still in dispute to some extent. It then follows that the reduced viability of homozygous thrum has been developed since the time when the dominance relations became fixed. Dominance is not a special property of the thrum gene in a dimorphic population, such as we now have, but is a relict of an earlier stage in the evolution of the genetic system of Primula.

This conclusion is in no way an unlikely one, as once a particular dominance relation has developed under the action of selection it will be maintained even in the absence of any further selection of the same type, provided that nothing supervenes to render advantageous a change in the opposite direction. We must then suppose that the dominance of thrum is such a relict of an earlier genetical system on which no back selection has been exercised.

The reduction in viability of homozygous thrum is directly attributable

to the action of the outbreeding mechanism which heterostyly and illegitimacy constitute. The population consists of pin and thrum plants, and effective pollination in any plant is always by pollen from an individual of the opposite kind. Thus the thrum plants will always be heterozygous and the **S** carrying chromosomes, or at least the sections immediately adjacent to the **S** locus, will be sheltered from the action of selection. Deleterious recessive genes can thus accumulate near **S** without their having any effect on the fitness of the individuals carrying this chromosome. But just as soon as an artificial selfing results in the production of **SS** thrums, the full effects of the genes will be seen in the reduction of the proportion of such homozygote that can be recovered. To express this in other words, the thrum homozygote is never produced naturally and the internal balance of polygenic combinations in the **S** carrying chromosome is poor, with the consequence that **SS** plants are at a disadvantage (Mather, 1941).

When the reduced viability of the thrum homozygotes is thus linked to the operation of the outbreeding mechanism it becomes clear that dominance of thrum over pin must have developed prior to the rise of outcrossing. Inasmuch as it must be supposed that the ultimate ancestors of Primula were not heterostyled, the breeding system has changed during the evolution of the genus. For the out-crossing mechanism must have developed in response to a rise in the optimum rate of inbreeding. Thus the ancestral populations had no especial means of encouraging outcrossing, or at least had a less efficient means of doing so, and there would be no bar to the production of all possible genotypes at the **S**, **s** locus. So there would, in such a population, be every opportunity for the development of dominance provided **s** were at a slight disadvantage as compared with **S**. In such circumstances **s** would be a comparatively rare recessive floating in an **S** population just as mutants are known to float in present-day populations of Drosophila and other organisms.

It is not necessary to suppose that the prototypes of pin and thrum were exactly like the plants of these kinds that we now observe. In fact it is highly unlikely that this would be the case. The pin plant would merely have to differ somewhat from thrum, morphologically and physiologically, in such a way that crossing between them would be relatively more frequent than selfing or crossing between two individuals of the same type. The advantage or disadvantage such crossed progeny would command, as compared with inbred plants, would vary with the optimum rate of outbreeding; this in turn would depend on the environment at the time. Initially the advantage of outbreeding must have been so small as to be outweighed by the innate disadvantage of the **s** allelomorph. But as soon as the changing environment raised the optimum outbreeding rate the extra hybridity of progeny, which had one parent pin, overcame the natural disadvantage of **s**, and this allelomorph spread in the population. At the same time modifiers strengthening the outbreeding action, i.e. genes at loci of the Primrose Queen and fertile double kind, would

be selected, and pin and thrum would take on the characters which they show to-day. Thus heterostyly and illegitimacy, as we know them, developed.

It should be noted, too, that this spread of pin and the strengthening of the outbreeding mechanism, leading to rigorous crossing between pin and thrum, would eliminate **SS** plants from the population with the consequences seen above. At the same time pin (**ss**) and the remaining thrum (**Ss**) would tend to diverge rather than converge in form and function, and there would be no tendency for back selection of the heterozygote towards the recessive homozygote. The dominance relations developed and fixed in the days prior to constant outbreeding persist after heterostyly has become the rule, while the **S** chromosome is accumulating deleterious recessives as a result of its sheltered state. Thus the apparent contradiction presented by dominance of the less viable allelomorph is resolved when the time factor is taken into account, and its resolution shows how the genetical mechanism of heterostyly and illegitimacy has arisen.

The change in the breeding system, under the influence of an increased hybridity optimum, is, as seen above, both in the direction of greater control and of increased outcrossing. But the advantage of increased hybridity is not necessarily permanent, and a second change in environmental conditions may place a premium on reduced variability and hence on the low hybridity which results from inbreeding. It is instructive to see how the outbreeding system can react to such a change.

The genetical situation resulting from the rise of heterostyly and illegitimacy consists essentially of two parts. There is first of all the switch mechanism at the **S, s** locus which determines whether an individual shall be thrum or pin, and secondly there exists a highly selected complex of modifying genes which determine that the plants will be good and efficient pins or thrums. Either part of the mechanism might be changed by a decrease in the optimum rate of outbreeding.

Let us first consider possible changes in the modifier complex. This system of genes has been selected for its action in balancing the morphological and physiological properties of pin and thrum. The only change which would be induced by a greater optimum of inbreeding is back selection towards the less highly specialized state which had existed earlier. This would demand that the original allelomorphs of many of the genes still floated in the heterostyled population. In other words such back selection would only be effective where the modifier complex were not too well adapted to outbreeding. In many Primula species the illegitimacy reaction is so strong that selfing and intercrossing of like plants gives no seed, and in such cases back selection of the modifier complex is difficult and very unlikely. In other cases where the species is not so highly adapted to outbreeding such a reversal is more likely and has indeed probably occurred in *P. sinensis*; though here, admittedly, the change was induced by artificial selection. Commercial seed raisers prefer plants which may be selfed, as this enables them to keep pure stocks with

relative ease. Indeed the standard practice with *P. sinensis* is, and has long been, to maintain stocks by selfing pin plants. Strains which do not easily self are not normally kept. The effect of this selection may be seen in Table VII, in which the relative fertility of illegitimate and legitimate crosses in present-day *P. sinensis* is compared with that recorded by Darwin in 1877 from his own and Hildebrand's experiments. The rise in fertility of illegitimate matings as time goes on is very strikingly shown by these figures, and there can be no doubt that the efficiency of the illegitimacy mechanism has been much reduced by back selection of the modifiers.

TABLE VII

		Hildebrand, 1864.			Darwin, 1877.			New Data†		
		Fls. fertilized.	Capsules set.	Aver. seeds/caps. fertilized.	Fls. fertilized.	Capsules set.	Aver. seeds/caps. fertilized.	Fls. fertilized.	Capsules set.	Aver. seeds/caps. fertilized.
Legitimate	(a) Ss × ss .	14	14	41	24	16	33	929	829	30·0
	(b) ss × Ss.	14	14	44	8	8	64	889	696	17·8
Illegitimate	(c) Ss × Ss.	53	47	16	20	13	23	2444	1956	15·7
	(d) ss × ss .	37	27	11	7	4	14	24770	17932	13·6
$\sqrt{\left(\dfrac{cd}{ab}\right)}$*			0·31			0·39			0·63	

* $\sqrt{\left(\dfrac{cd}{ab}\right)}$ is taken as a measure of the fertility of illegitimate, relative to legitimate, pollinations as it compares the behaviour of the parents of the illegitimate matings with their respective performances as both male and female in legitimate matings.

† The low number of seeds given by ss females is perhaps due to pin plants carrying more recessive genes than the thrums, as a result of the nature of the breeding experiments of which they formed part.

The switch mechanism at the S, s locus offers the possibility of a very different adjustment to changed breeding conditions. In a heterostyled Primula species the S, s gene controls three differences, viz. the height of the anthers, the height of the stigma, and the illegitimacy behaviour. In ss plants the alternatives to these three occur together. It has, however, been found by Ernst (1933) that other allelomorphs at this locus can give different combinations of the three differences. This is not unexpected in view of the behaviour of such genes as scute and yellow in Drosophila, where allelomorphs show various combinations of specific effects on different parts of the organisms.

Changes of this type at the switching locus S, s allow of effective adaptation to altered breeding conditions. A common substitution is that of homostyly for heterostyly. An allelomorph which reverses the relation between stamens

and pistil while maintaining the association of each with its characteristic illegitimacy reaction arises. This gives either long or short homostyle types
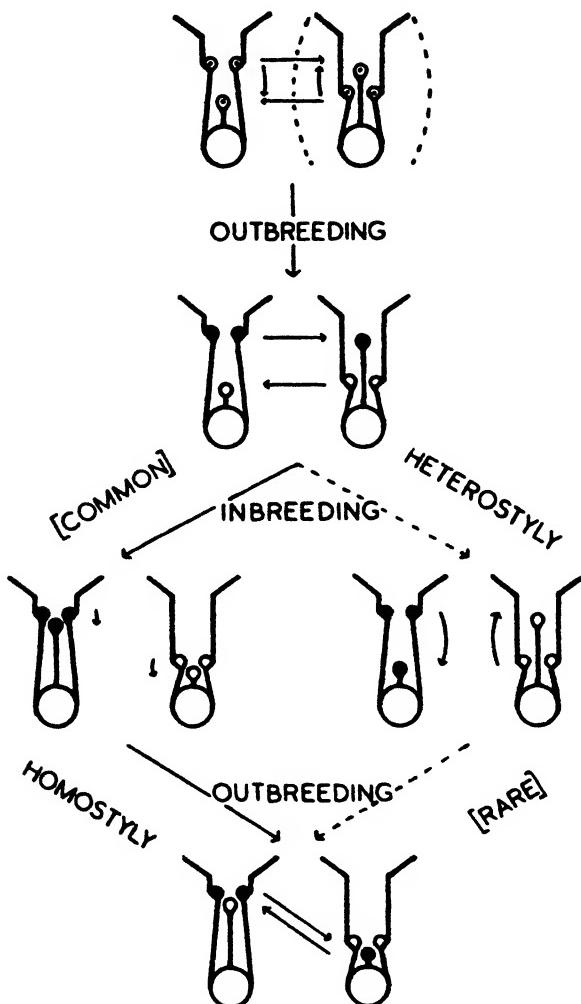
FIG. 1. The origin of heterostyly and homostyly in Primula. Legitimate pollinations are indicated by arrows and also by shading of the anthers and stigmata. The brackets round the pin plant at the top of the diagram indicate that it was supposedly a rare recessive variant in an otherwise thrum population. The inbreeding heterostyled type has not yet been observed, and the outbreeding homostyle is rare. For further details see the text.

which will be characterized by their ability to produce progeny as a result of self-pollination. Such an allelomorph will enjoy a marked selective advantage where a high inbreeding optimum is the rule, and will indeed completely replace the heterostyle allelomorphs in such cases.

It should be noticed that this type of adaptation for inbreeding is not a

reversion towards the pre-heterostyle state. Homostyly is just as much a controlled breeding system as heterostyly, but leads to inbreeding as opposed to outbreeding. It is, in fact, a natural step in the sequence of events initiated by the development of the outbreeding mechanism. The illegitimacy mechanism which could only develop under the stimulus of outbreeding advantage is, when established, adaptable to give a controlled inbreeding system, and the more rigorous the selection of illegitimacy to outbreeding conditions the more efficient it is as an inbreeding mechanism when the switching genes are changed. Furthermore, a combination of homostyly and heterostyly, such as has been observed in *P. vulgaris* by Crosby (1940), can presumably give almost any intermediate rate of outbreeding or inbreeding.

Homostyly results from a reversal of the relations of stamen and pistil length, but inbreeding could also be achieved by an allelomorph at the **S, s** locus which reversed the relations of illegitimacy with, say, the pollen while maintaining it as before on the female side, and which maintained the original floral morphology (see Fig. 1). This has not been observed in nature, though Ernst has found in *P. viscosa* and *P. hortensis* outbreeding homostyle types which would arise from ordinary homostyle by a change of this kind. These types are rare in the wild, and there is no reason to expect that they should be frequent, as the common forms of heterostyly and homostyly are sufficient to ensure outbreeding and inbreeding respectively, so rendering redundant any further simplification. Thus in Fig. 1 the first three stages are the important ones. The other two are possible variations, one having been found, the other not, which are of doubtful adaptive value and which may therefore be expected to be rare in the wild.

Summary

Heterostyly involves both morphological differences in flower structure and physiological differences in pollen behaviour in the two kinds of cross, legitimate and illegitimate. The illegitimacy reaction and not the morphological difference is the bar to inbreeding.

In *P. sinensis* thrum is dominant to pin and yet the thrum homozygote is less viable than thrum heterozygote or pin. This apparent contradiction can only be resolved when the time factor in evolution is taken into account, and it is supposed that pin arose as a deleterious mutant first, and that heterostyly was developed later. When outbreeding became advantageous the initial unfitness of pin was outweighed by its encouragement of crossing. It then spread, and simultaneously modifiers strengthening the illegitimacy reaction were accumulated by selection until heterostyly was fully developed. Such modifiers are known in this species. Homozygous thrums are never produced by heterostyled populations, and this sheltering of the S carrying chromosome results in its picking up deleterious recessive genes, which reduce the viability.

Homostyly must be due to a change in the switch mechanism of the **S, s** locus which results in the illegitimacy reaction, developed by its encouragement of outbreeding, being used to determine controlled inbreeding.

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Symbiosis of Leguminous Plants and Nodule Bacteria

I. Observations on Respiration and on the Extent of Utilization of Host Carbohydrates by the Nodule Bacteria

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With one Figure in the Text

INTRODUCTION

IT is obvious that, as heterotrophic organisms with regard to carbon, the bacteria which inhabit the root nodules of leguminous plants must derive organic materials from the host plant for use in their growth and respiratory processes. With these particular bacteria there is perhaps (though not necessarily) a special requirement of such material for the provision of energy which may be necessary in the process of fixation of nitrogen. Any information that can be obtained regarding the extent to which host materials are utilized by the nodule bacteria for these purposes will be of value in several connexions, including the elucidation of the fixation process itself. It seems probable, and for the purpose of the present communication it is convenient to assume, that the materials used by the bacteria will consist mostly of carbohydrates or their immediate derivatives.

One method by which such information has been sought in the past is that used by Christiansen-Weniger (1923) and by Allam (1931). Their experiments receive critical consideration from Allison (1935). This method (though modified somewhat by Allam) consists essentially in the determination of the amount by which the dry weight of plants grown asymbiotically on combined nitrogen exceeds that of nodulated plants grown on symbiotic nitrogen but otherwise exposed to similar conditions. This difference is taken to be an indication of the amount of organic material of host origin utilized by the nodule bacteria in their own metabolism. Reference to results obtained by this method will be made later.

Since it is probable that most of the carbohydrates appropriated by the bacteria will actually be utilized in respiratory processes (using the term in the widest sense), a second method of investigating the consumption of carbonaceous materials by the nodular bacteria lies in observations on the intensity of respiration, as indicated by the intake of oxygen or the output of carbon dioxide. Newton (1923), in another connexion, reported that the amount of carbon dioxide evolved per unit dry weight of pea roots (including nodules) was more than twice that from roots of barley, but he did not consider the extent to which the nodules were responsible for this difference. Reinau (1927) showed that the evolution of carbon dioxide from the surface of soil in which leguminous plants were growing was usually appreciably higher than from soil carrying other plants. The following figures may be quoted from Reinau (gm. carbon dioxide per sq. metre of soil surface per hour):

| Clover | . | . | 0·558 | Mustard . | . | 0·218 |
| Seradella | . | . | 0·305 | Rye | . | . | 0·285 |

Reinau concluded that these differences were due to the root nodules of the legumes being the centre of active katabolism. Allison (1935) has, however, pointed out that other factors may have contributed to the increased evolution of carbon dioxide from the soil carrying legumes. It was consideration of the possibilities of Reinau's method that led to the present author's experiments. It may be noted here that from the data of Reinau, together with values from various other sources, Allison (loc. cit.) arrived at the provisional conclusion that the nodule bacteria utilize not more than 3–6 per cent. of the total carbohydrate synthesized by the leguminous host plant.

In recent papers by Allison, Ludwig, Hoover, and Minor (1939, 1940) and Allison, Ludwig, Minor, and Hoover (1940), published while the present author's experiments were in progress, a report is presented of extensive observations on the respiration of detached nodules of various leguminous plants, a Warburg apparatus being employed. Among other findings they conclude, contrary to a view which they point out to have been held frequently in the past, that the nodule is not the centre of any specially intense respiratory processes, since, on the basis of oxygen absorbed per unit dry weight, the rate of respiration of nodules was not found to be appreciably greater than that of detached roots of the leguminous plants. Bearing in mind the small relative weight of the nodules, Allison et al. find in their results confirmation of the view that the nodule bacteria utilize only a very small proportion of the total carbohydrate synthesized by the host plant.

In the experiments to be described in the present paper, determinations of the respective rates of evolution of respiratory carbon dioxide from nodules, roots, and tops of intact leguminous plants have been made. From the results it is possible (so far as carbon dioxide-evolution is a reliable index—see later) to compare the utilization of carbohydrate in the respiration of the nodules and bacteria with that in the respiration of the other parts of the plant. Certain conclusions are possible also on the relation between this bacterial

consumption of carbohydrate and the processes of photosynthesis and fixation of nitrogen. In these respects the observations are somewhat more complete than those of Allison et al., since those investigators examined the respiration of selected nodules and root fragments only, and their data do not permit of calculations in respect of entire plants or of fixation of nitrogen, except in a very general way.

The use of detached organs for observations on respiration, the procedure adopted by Allison et al., permits of the utilization of more precise methods for measuring gaseous exchange. There is, however, the possibility, appreciated by those authors, that the isolation of particular organs will affect their activities to a certain extent. In the case of the nodule most investigators have concluded that detachment results in the cessation of the process of fixation (see Allison, Ludwig, Hoover, and Minor, 1939; Wilson, 1937; Wyss, Burris, and Wilson, 1939; Virtanen, 1938)[1]; it seems possible that this would be accompanied by changes in other nodular activities. At the same time the fact that the respiration of detached nodules and roots remained fairly constant over periods of several hours in the experiments of Allison et al. indicates that isolation had at least no progressive effect on respiration. In observing the gaseous exchange between the atmosphere and an organ still attached to the plant, the possibility has to be borne in mind that the actual evolution or absorption of a gas produced or utilized in that organ may occur partly from or at the surface of some other organ of the plant. Thus it has been suggested that some of the carbon dioxide formed by the respiration of root cells may, instead of being evolved from the surface of the roots, be carried by the transpiration stream to the aerial parts of the plant (Cerighelli, 1925). This possibility is mentioned again in the next section. Another difficulty in working with intact plants, peculiar to the present problem, is that the carbon dioxide evolved from the nodules cannot then be measured separately from that produced by the roots, and an indirect method, which is explained later, has to be adopted in order to determine the respective contributions of roots and nodules to the total evolution of carbon dioxide from the nodulated root systems. Despite these various difficulties attached to the method of experimentation adopted in the present work, it seems to be desirable that some data based on observations on intact plants should be available, provided of course that methods of reasonable accuracy can be devised for making such observations.

It is realized that the rate of evolution of carbon dioxide is not in all cases a reliable indication of the rate of consumption of carbohydrate substrate in the respiratory process, for the reason that a varying proportion of the carbon present in the original substrate may actually emerge as carbon dioxide. The need for caution in this respect is indicated in the first place by the results of Allison et al. (loc. cit.), for they found that although the respiratory quotient in small leguminous roots was slightly below 1, in the case of nodules higher

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[1] Virtanen reports fixation in excised nodules in the presence of oxalacetic acid, but Wyss, Burris, and Wilson were unable to confirm this.

values ranging up to 2 were obtained. Apparently some anaerobic respiration was in progress within the nodules, accompanied presumably by the formation within the tissues of carbonaceous products other than carbon dioxide.[1] In this event the amount of carbon dioxide produced would not be a fully reliable index for comparing the utilization of carbohydrates by root and nodule tissues. The results of Georgi and Wilson (1933) may also be noted. Working with pure cultures of the nodule organism, under aerobic conditions, they found that with pea, clover, and alfalfa organisms the percentage of the carbon of the glucose consumed that appeared as carbon dioxide was of the order of 60–80, rather lower figures being obtained with the soya bean organism. The rest of the carbon was considered to pass into the form of gum. It is uncertain to what extent these findings can be extended to nodular bacteria.

METHODS

The experiments were carried out during the summers of 1939[2] and 1940 on plants of soya bean grown in water (or solution) culture in the greenhouse. The general principle of the procedure was as follows. It was arranged that two groups of plants were available for experiment, one group bearing nodules, the other group being kept free of infection by the nodule organism. The former plants were supplied with a nitrogen-free culture solution, while to the culture solution of the non-nodulated plants amounts of combined nitrogen estimated to produce plants of similar development and vigour to the nodulated plants were added at intervals. In other respects the treatment of the two groups was identical. Observations on rates of respiration were made at a single stage of development of the 1939 plants, at two stages in the 1940 experiment. For the purpose of these observations the plants were transferred to a laboratory which could be maintained at a temperature of 19–21° C. for the required period. In the case of the nodulated plants determinations were made of the rate of evolution of carbon dioxide by the whole root system, inclusive of the nodules, and some observations were also made on the respiration of the tops of the plants. In the case of the plants receiving combined nitrogen the root respiration alone was determined, these observations being concurrent with those on the roots and nodules of the first group. As soon as an adequate number of observations on respiration had been made, the plants concerned were harvested and the dry weights of roots, tops, and nodules (where present) ascertained. By proceeding on the basis that respiration per unit dry weight of root tissue was similar in the two sets of plants (and evidence in favour of this is presented later), it was then possible to calculate the

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[1] Virtanen, Nordlund, and Hollo (1934) have studied the fermentative action on glucose of crushed nodules and of the nodule organism in pure culture, and report the formation of lactic, butyric, and acetic acids, and ethyl alcohol, in addition to carbon dioxide and hydrogen.

[2] A brief notice concerning the 1939 experiment has already appeared (Bond, 1939).

respiration of the roots of the nodulated plants, and so arrive at the nodule respiration by difference.

Cultivation of plants.

The 'Manchu' variety of soya bean was used, seeds being kindly supplied by Dr. W. J. Morse, United States Bureau of Plant Industry, Washington. An excess of seeds of closely similar weight, previously surface-sterilized, was sown on May 15 in both years in large dishes of moistened sand, the seeds in the case of certain dishes being inoculated with an efficient strain of the soya bean nodule organism (Wisconsin strain 505) immediately prior to planting. At the stage when the first two foliage leaves were unfolding, uniform seedlings were selected and transplanted into water culture. A few macroscopic nodules were present on the roots of the inoculated plants at this stage. For water culture use was made of glass jars of capacity $2\frac{1}{2}$ litres, fitted with 7-hole rubber stoppers, five plants being grown per jar. Crone's nitrogen-free solution was initially supplied to all jars: this solution was used successfully by Bryan (1922) in water-culture experiments with nodulated soya beans. In the present experiments the solution was made up with tap water, and only the clear supernatant solution, siphoned off after the mixture had been shaken and allowed to stand for twenty-four hours, was used. A solution containing traces of B, Mn, Zn and Cu (Loomis and Shull, 1937) was also supplied at the rate of 10 c.c. per jar. As soon as appreciable root elongation had occurred the level of the solution in the jars was reduced until only 2 litres was present in each, with the result that the more basal parts of the root system were exposed to air. This was in view of the findings of some previous investigators (see Allison, 1935) that better and more effective nodulation is then secured than if the root systems are totally immersed. A further quantity of inoculum was added to those jars containing plants already inoculated at sowing. Through one of the remaining two holes in each stopper a glass tube passed to the bottom of the jar and was inserted there into a rectangular aerator of porous porcelain, measuring $5 \times 2\cdot5 \times 2\cdot5$ cm., of a type used for aquaria (see Fig. 1). By means of this arrangement a liberal aeration of each culture was effected for twelve hours each day, air being supplied by an electrical compressor. The jars were wrapped with opaque paper, and in later stages of growth the plants were supported by means of thin stakes tied to the sides of the jars.

Precautions had, of course, to be taken to preclude infection of the uninoculated plants by the nodule organism. All materials and solutions were sterilized before use, and the air supply was filtered through cotton wool before it reached the plants. As a result of these and other precautions none of the uninoculated plants developed nodules.

The culture solution in the jars was renewed every fourteen days. At the first date of renewal, 40 mg. of combined nitrogen, in the form of a sodium

nitrate solution, was added to each jar of uninoculated plants, and a similar dose was supplied at each subsequent date of renewal.
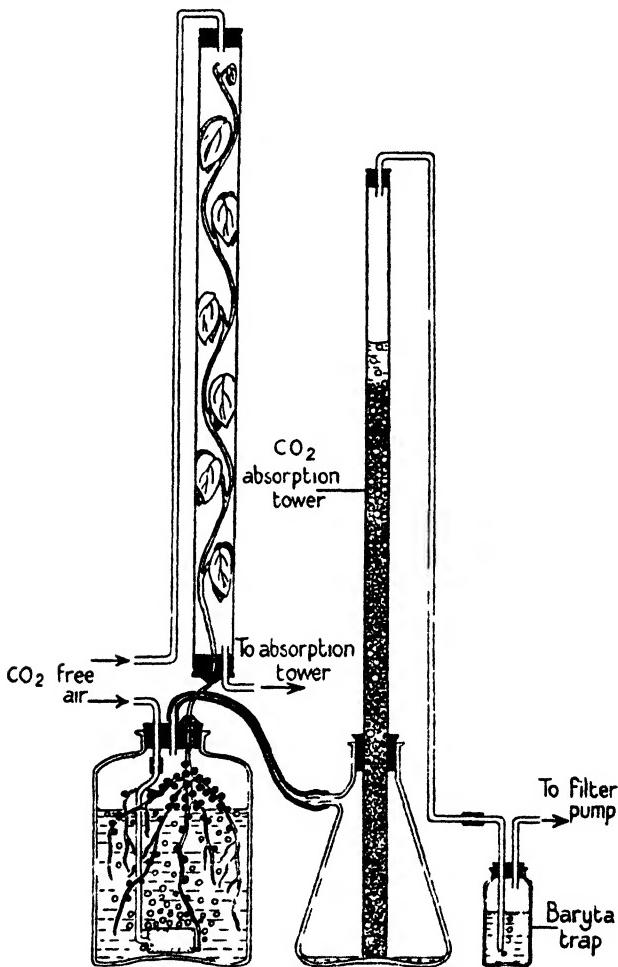
Apparatus used in the measurement of the evolution of carbon dioxide from the roots and nodules and the tops of the experimental plants. Actually five plants were grown in each jar, while the tops of two were enclosed together in the tube used for the measurement of respiration. For further description see text.

Determination of respiration.

It will be convenient to consider first the determination of carbon dioxide-evolution from root systems, including nodules where present. In preparing for these determinations the holes in the stoppers through which the plant stems passed were sealed up with modelling clay ('plasticine'). Each jar was then inserted into a gasometric train of usual type, whereby a vigorous current of air, freed of carbon dioxide by soda lime, could be drawn through the

culture jars and thence into absorption towers of the Reiset type, 85 cm. in height, and partially filled with glass beads, each containing initially 100 c.c. of N/20 baryta (see Fig). In the 1940 experiment ten trains were operated concurrently, allowing of simultaneous estimation of the root respiration of four jars (20 plants) of non-nodulated plants, and of the combined root and nodule respiration of the same number of nodulated plants. Two control trains (no plants) were included. The flow of air through each jar was of the order of 8–10 litres per hour. Some variation in this respect was necessary because of differences in porosity of the porcelain aerators, some emitting fewer, larger bubbles than others under a given pressure difference. In practice the air current through each jar was adjusted until there was profuse aeration of the solution, with a minimum proportion of dead space through which air bubbles were not passing.

Prior to the actual commencement of a determination, carbon dioxide-free air was drawn through the circuits, without the towers in position, for a period of $1\frac{1}{2}$–2 hours, and during this time tests for air-tightness of the circuits were carried out, under tensions equal to or greater than those normally obtaining within the apparatus, these latter being of the order of 10 cm. of mercury. Occasional tests showed that at tensions double those normally prevailing the plasticine seals round the plant stems were still satisfactory.

The duration of a determination of root respiration varied from three to six hours. At the conclusion of the selected period the contents of each absorption tower were titrated with N/20 HCl, after having been washed down into the flask by means of 200 c.c. of freshly boiled water. The method by which nodule respiration was calculated from these determinations is explained in the next section.

In order to test the efficiency of the towers in absorbing carbon dioxide, on two particular occasions when root respiration was being determined, a second tower was introduced in series with the usual one, so as to measure any leakage past the latter tower. The results indicated that the percentage of carbon dioxide that was absorbed by the first tower amounted to 97·7 and 97·4 respectively in the two tests.

Mention has already been made of the possibility, advocated especially by Cerighelli (1925) on the basis of his experimental observations, that a proportion of the carbon dioxide produced by respiration of root cells is not actually evolved from the root surface but is carried up in the transpiration stream to the leaves. Löweneck (1930), using more conclusive methods, was unable to demonstrate any influence of the rate of water intake by roots on the evolution of carbon dioxide from those organs, and the experiments of Henderson (1934) also provide no evidence of such influence. Cannon (1932) has pointed out that Cerighelli's results can be explained alternatively on the basis of a downward diffusion of oxygen through the plant. It is, however, perhaps worth pointing out that any error arising in the present experiments as a result of internal transport of carbon dioxide would be in respect of the figures for

respiration of roots rather than in those for the nodules, since it is improbable that there is any appreciable flow of water through the latter, and in any case the majority of the nodules in the present instance were above the level of the solution. Actually the fact that the values for root respiration obtained in these experiments are of the same order as those of other workers using detached roots of the same species of plant (see later) suggests that internal transport of carbon dioxide did not attain serious proportions. It may also be stated that there was no sign of any development of non-symbiotic bacteria or of other micro-organisms in the culture solutions.

Ideally the determinations of the respiration of tops (this term includes all parts of the plant above the stopper of the culture jar) would have been concurrent with those on the roots, but the time required for assembling the apparatus precluded this. Actually the measurements of top respiration were made as soon as possible after root respiration had been established. The tops were enclosed in long glass tubes, measuring 100×2.5 cm. (Fig. 1). Two plant tops were inserted into each tube, and by means of a slit the stems could be introduced and sealed with plasticine into one of the holes in the lower stopper. The whole of the experimental shoots except a short length of stem at the base was thus enclosed in the tube, which was wrapped in black paper and connected up to a train otherwise similar to that used for root respiration measurements. Carbon dioxide-free air was drawn through the apparatus for $1\frac{1}{2}$–2 hours, during which tests for leaks were effected; this period would also allow of the partial or complete disappearance of any effects on respiration of the manipulation of the leaves involved in introducing the shoots into the tube (Audus, 1939). The rate of flow of air was similar to that employed for the roots, and since the volume of a tube was approximately 2 litres, its atmosphere was completely changed every fifteen minutes or so. The duration of each determination was six hours.

As indicated, as soon as the values for respiration had been established the plants were harvested, and tops, roots, and nodules dried to constant weight at $95°$ C. Total nitrogen contents were subsequently determined by means of Kjeldahl analyses on samples of the dry matter.

EXPERIMENTAL RESULTS

Reference will first be made to the general growth and development of the plants utilized in the experiments. The data obtained when the plants were harvested are presented in Table I. In the preliminary experiment of 1939 only three jars of nodulated plants (i.e. a total of fifteen plants) and two jars containing non-nodulated plants supplied with nitrate were available. They were used for a single series of determinations of respiration at the flowering stage. In 1940 eight jars (forty plants) of each type were available, four jars of each type forming the basis of the first series of determinations of respiration at a stage shortly before flowering, while the remaining jars were used

in a second series of determinations at a later stage when young pods were present.

The growth of the plants was satisfactory in all cases. It will be seen from Table I that there was abundant nodulation in the case of the inoculated plants. The majority of the nodules were situated on the more basal regions of the roots, which, as noted already, were exposed to air, or on roots only slightly submerged; some more deeply submerged nodules were also present. In size the nodules ranged up to a diameter of 8 mm. As confirmation of the satisfactory nodulation it may be noted that other investigators, as reported by Allison (1935), have found that the dry weight of nodules on annual legumes grown under very favourable conditions is usually about 7 per cent. of the total dry weight of the whole plant. The corresponding figures for the plants used in the present experiments were as follows: 1939, 7·5 per cent.; 1940, first harvest, 8·7 per cent.; second harvest, 9·2 per cent. It may be noted that in the case of jar 1 of the 1940 experiment (Table I) an unusually large number of nodules developed, with an average size below normal. The reason for this was not apparent.

Examination of the figures for nitrogen content of the nodulated plants (which received a nitrogen-free culture solution) indicates that there was satisfactory fixation of nitrogen. The actual amount of nitrogen fixed at any harvest is less than the nitrogen content of the plants by approximately 50 mg., the amount derived by the plants from the original seeds. On the other hand, in the case of the plants of the second harvest in 1940, a certain amount of nitrogen, estimated at 30 mg., is to be added on account of leaves falling from the plants between the first and second harvests. After making these adjustments, it appears that the fixation by the time of the second harvest in 1940 amounted to about 300 mg. for five plants. It may be noted that no excretion of fixed nitrogen has been observed from nodulated soya beans growing in sand culture at this station (Bond and Boyes, 1939), and this, coupled with the inability of Virtanen (1938) to secure appreciable excretion in water cultures (even from peas, which give good excretion in sand culture under Virtanen's conditions), makes it fairly certain that there was no loss of fixed nitrogen in this way in the present experiments.

Reference to Table I shows that the non-nodulated plants attained a greater dry weight than the nodulated plants, especially in respect of the root systems, which were much more extensive. The leaves of the former plants had not, however, such a deep green colour as those of the nodulated group. By the second harvest in 1940 a total of approximately 250 mg. of nitrogen had been supplied to the non-nodulated plants as sodium nitrate. Examination of the nitrogen contents of the plants indicates, after adjustments on account of seed nitrogen and fallen leaves (see above), that practically all the nitrogen supplied had been absorbed. It will be noted that the nitrogen contents of nodulated and non-nodulated plants were quite similar, especially if the nodule nitrogen is first subtracted from the former. Since, as has been pointed

TABLE I

Dry Weight (gm.) and Total Nitrogen Content (mg.) of the Plants used in the Experiments (All data are for five plants)

Date.	Jar No.	Type.	Tops. Dry wt.	Tops. N content.	Roots. Dry wt.	Roots. N content.	Nodules. No.	Nodules. Dry wt.	Nodules. N content.	N content of complete plants.
1939 Harvest Aug. 29	1	Nodulate	6·03	115·6	1·57	29·2	190	0·785	34·6	179
	2		6·60	135·9	2·28	42·4	183	0·677	29·8	208
	3		5·90	89·6	1·69	31·5	225	0·487	21·5	143
		Av.	*6·18*	*113·7*	*1·85*	*34·4*	*199*	*0·650*	*28·6*	*177*
	1A	Non-nodulate	12·05	206·7	3·84	68·1	—	—	—	275
	2A		8·35	143·3	3·26	57·8	—	—	—	201
		Av.	*10·20*	*175·0*	*3·55*	*63·0*	—	—	—	*238*
1940 First stage Harvest July 29	1	Nodulate	4·94	110·0	1·00	19·9	(574)	0·454	23·9	154
	2		5·13	117·6	1·17	23·3	195	0·493	26·0	167
	3		6·80	175·2	1·13	22·4	170	0·675	35·6	233
	4		5·79	129·2	1·08	21·5	137	0·522	27·5	178
		Av.	*5·67*	*133·0*	*1·10*	*21·8*	*167*	*0·536*	*28·3*	*183*
	1A	Non-nodulate	6·79	120·1	2·22	40·8	—	—	—	161
	2A		6·79	120·1	1·81	33·3	—	—	—	153
	3A		7·39	130·8	2·21	40·6	—	—	—	171
	4A		7·84	138·8	2·32	42·7	—	—	—	182
		Av.	*7·20*	*127·5*	*2·14*	*39·4*	—	—	—	*167*
1940 Second stage Harvest Sept 7	5	Nodulate	12·01	267·8	1·68	33·0	368	1·366	69·6	370
	6		7·02	149·4	1·54	30·6	280	1·016	51·7	232
	7		13·48	302·9	1·82	35·8	262	1·535	78·2	417
	8		9·99	184·8	2·08	40·9	220	1·137	58·0	284
		Av.	*10·63*	*226·2*	*1·78*	*35·1*	*283*	*1·264*	*64·4*	*326*
	5A	Non-nodulate	14·24	172·6	4·36	82·9	—	—	—	256
	6A		15·16	181·8	4·81	91·4	—	—	—	273
	7A		13·76	not determined	4·31	not determined	—	—	—	—
	8A		13·80	,,	5·15	,,	—	—	—	—
		Av.	*14·24*	,,	*4·66*	,,	—	—	—	—

Note. The figures for nitrogen content are based on analyses on the combined dry matter from replicate jars, except in the case of the tops of nodulated plants, where analyses on dry matter from individual jars were carried out.

out above, the dry weights of the non-nodulated plants were considerably greater than those of the nodulated group, it follows that the percentage of nitrogen within the tissues of the former plants was lower. The extent of the difference is indicated by the following figures for percentage nitrogen in the dry matter of the 1940 plants:

		1st harvest.	2nd harvest.
Nodulated plants	Tops	2·33	2·11
	Roots	1·99	1·97
	Nodules	5·27	5·09
Non-nodulated plants	Tops	1·77	1·21
	Roots	1·84	1·90

The greatest difference is in respect of the tops. Experience gained in other experiments indicates that nitrogen supply was limiting the growth of the non-nodulated plants, inasmuch as they would have made more growth had they received a larger supply of nitrate. The fact that although they had a similar content of nitrogen the nodulated plants showed lower dry weights than the non-nodulated group is doubtless related to the consumption of a proportion of carbohydrate by the bacteria associated with the former plants (see later). It will be observed that in the 1940 experiment there was considerably more variation from jar to jar in respect of dry weight and nitrogen content in the nodulated plants than in the non-nodulated group. Such variation among replicate pots of plants depending on symbiotic nitrogen is a matter of common experience, and is doubtless indicative of the greater number of variables operating in the growth of such plants.

The results obtained in the determinations of respiration will now be considered. They are expressed in two ways. In the first instance they are presented in terms of mg. of carbon dioxide produced per hour per gm. dry matter of roots, nodules, or tops. Secondly they are expressed on an absolute basis in terms of mg. of carbon dioxide produced per hour by the whole root system, by all the nodules present on a plant, or by entire tops. Although it is essentially in this latter form that the data were originally obtained, it is convenient to consider first the figures on the unit dry weight basis, since this facilitates comparison of results obtained with different plants or on different occasions.

It may first be repeated that all measurements of respiration were effected within the temperature range of 19–21° C. Also at this point further reference will be made to the method of calculating the respiration of nodules. As indicated on p. 316, in the observations on the nodulated plants the combined respiration of roots and nodules was determined. In order to calculate the respective contribution of roots and nodules to this total it is necessary to make use of a value for the respiration of unit dry weight of root tissues established in parallel observations on the non-nodulated plants. By this means the respiration of the roots of the nodulated plants can be calculated, and the respiration of the nodules then found by difference. A typical calculation, relating to the determinations of root and nodule respiration of July 24, 1940 (see Tables IV and V), will make the matter clearer.

The total carbon dioxide evolved from the root systems including nodules of jar 1 was 21·48 mg. over a period of 4·5 hours, or 4·77 mg. per hr.

Simultaneous observations under the same conditions on the roots of four jars (Nos. 1A–4A) of non-nodulated plants gave an average of 2·55 mg. carbon dioxide per hr. per gm. root dry matter.

Since the dry wt. of the roots of the nodulated plants of jar 1 at the harvest immediately following was found to be 1·00 gm., the total production of carbon dioxide by these roots during the experimental period, if their respiration per unit dry wt. was equal to that of the non-nodulated roots, was 2·55 mg. per hr.

Thus the production of carbon dioxide by the nodules of jar 1 amounted to $4·77 - 2·55 = 2·22$ mg. per hr. Since the dry wt. of these nodules was 0·454 gm., nodule respiration in terms of mg. carbon dioxide per hr. per gm. dry wt. $= 4·90$.

Obviously the accuracy of this method of arriving at nodule respiration depends on how nearly the respiration per unit dry weight of the two types of roots was equal. In the first place it seems probable that the respiration of roots the nutrition of which differs only in respect of the source of nitrogen will be of the same order, especially in view of the finding of Allison, Ludwig, Minor, and Hoover (1940) that the rates of respiration of detached roots from different species of leguminous plants were much the same. More precise evidence is provided by observations kindly made by Dr. G. F. Asprey on detached roots from the present author's experimental plants. The respiration of comparable portions of roots from nodulated and non-nodulated plants was determined in a modified Barcroft apparatus designed by Asprey, in which the carbon dioxide is measured by an electrical conductivity method. The determinations were carried out on roots taken on different dates while the plants were in the flowering stage, and the results for three samples of roots from the non-nodulated plants, in terms of mg. carbon dioxide/hr./gm. dry wt., were 3·42, 4·80, 4·82, the average of these being 4·35. The corresponding results for three samples of roots from the nodulated plants (the selected roots being free of nodules) were 3·76, 4·50, 4·56, with an average of 4·27. Each figure is based on the examination of the root sample for a period of several hours, during which the respiration remained steady. It appears from these results that the respiration of the two types of roots was quite similar, so that the above method of arriving at nodule respiration is justified. It is not claimed that the method is one of extreme accuracy, but provided that the observations are based on an adequate number of plants it seems probable that the results will be reasonably correct. Asprey's figures are considerably higher than those obtained in the author's own observations on the roots of the non-nodulated plants (see later), but this is to be expected since Asprey examined only the younger, more active parts of the root system, while he also worked at a higher temperature $(25°\,\text{C.})$.

Tables II and III present the figures for respiration per unit dry weight of the roots of non-nodulated plants and the nodules of the inoculated plants obtained in the preliminary 1939 experiment, when the plants were at the flowering stage. In the observations of the first two days on the non-nodulated plants the gas currents from the two jars were combined and led into a single absorption tower. A similar procedure was followed with the nodulated plants, a single observation only being made on the third jar. The indication of these preliminary results is that the respiration per unit dry weight of tissue was about three times as intense in the nodules as in the roots.

In the more extensive experiment of 1940 the first series of observations involved measurements of respiration of nodules, roots, and tops. The figures

TABLE II

1939 Experiment. Respiration (mg. CO_2 per hr. per gm. dry matter) of the Roots of the Non-nodulate Plants at the Flowering Stage

Jar.	Aug. 23.	Aug. 25.	Aug. 28.
1A	} 1·57	} 1·63	1·79
2A			1·14

Mean 1·56.

TABLE III

1939 Experiment. Respiration (mg. CO_2 per hr. per gm. dry matter) of the Nodules of the Inoculated Plants at the Flowering Stage

Jar	Aug. 23.	Aug. 25.	Aug. 28.
1	} 5·32	} 3·63	3·60
2			5·55
3	—	8·13	—

Mean 5·02.

TABLE IV

1940 Experiment. Respiration (mg. CO_2 per hr. per gm. dry matter) of the Roots of the Non-nodulated Plants at a Pre-flowering Stage

Jar.	July 22.	July 23.	July 24.
1A	2·11	2·26	2·79
2A	2·36	2·79	2·40
3A	1·68	2·31	2·49
4A	2·50	2·35	2·51
Mean	2·16	2·43	2·55

Mean for the three days = 2·38.

for the roots of the non-nodulated plants are given in Table IV, and it will be noted that determinations were made on three successive days on four jars containing a total of twenty plants. The average for the first day was somewhat lower than on the following days, while there was also considerable variation between the different days in respect of the respiration of individual jars relative to the others. Some variation in results was expected, since it was not possible to exercise close control over temperature and other factors during and prior to the periods of the determinations.

The figures for nodule respiration at this stage are given in Table V, and are again based on the examination of twenty plants. It will be observed that here also there was considerable variation in the results from day to day. An unexpected feature appears on the first day, when a low figure for root respiration was accompanied by the highest figure for nodule respiration. It would be expected that any factor (at least an external one) productive of a low root respiration would have a similar effect upon nodule respiration. Since the values for nodule respiration were obtained by difference, it is clear that if for any reason the figure for root respiration employed in the calculation under-

estimated the actual respiration of the roots of the nodulated plants, then an erroneously high figure for the nodules would result. Such an under-estimate could arise (*a*) through some experimental error in the determination of the root respiration of the non-nodulated plants, which there is no reason to

TABLE V

1940 Experiment.   Respiration (mg. CO_2 per hr. per gm. dry matter) of the Nodules of the Inoculated Plants at a Pre-flowering Stage

Jar.			July 22.	July 23.	July 24.
1	.	.	7·45	3·95	4·90
2	.	.	7·11	5·56	6·53
3	.	.	7·46	4·91	6·48
4	.	.	9·10	6·71	8·12
Mean		.	7·78	5·28	6·51

Mean for the three days = 6·52.

suspect, or (*b*) as a result of an actual inferiority of root respiration in these plants on this particular day. There is the other possibility that the figures correctly reflect an actual fluctuation in nodule respiration, due to factors not readily recognized and perhaps of an internal nature. It may be noted that Allison et al. (1940) emphasize the great variation that they encountered in the respiration of apparently similar nodules. They refer, however, to variation between different samples, whereas in the present experiments there is variation between observations on successive days on the same samples. The experience of Allison et al. does not, however, preclude the occurrence of this latter type of variation in their material.

On the basis of the averages for the three days it appears that the nodule respiration per unit dry weight was rather less than three times as intense as that of the root tissues. The figures for respiration of both organs are higher than in the 1939 experiment, probably due in part to the observations being made at an earlier stage in the development of the plants.

Determinations of the respiration of the tops were made shortly afterwards on some of the nodulated plants used in the above observations. Six determinations were carried out, each involving a different pair of tops, so that twelve plants, selected as typical out of the total of twenty used in the nodule determinations, were examined for top respiration. The results on a unit dry weight basis are presented in Table VI, and it will be noted that the figures are lower than those for roots and nodules. It was obvious at the time that the variations between the results of individual determinations were due principally to differences in the nutrition and vigour of the tops used.

A second series of determinations was carried out some five weeks later on the remaining cultures of the 1940 experiment. By this time young pods were present, and the lower leaves were yellowing and falling. The root systems were still of very healthy appearance and there was no decay of nodules. It has already been pointed out that by this time rather large differences had

TABLE VI

*1940 Experiment. Respiration (mg. CO_2 per hr. per gm. dry matter) of
Tops (leaves and stems) of the Nodulated Plants at the Pre-flowering Stage.
Different Tops used on the two Days*

No.			July 26.	July 29.
1	.	.	1·64	1·17
2	.	.	1·44	1·47
3	.	.	1·28	1·47
Mean		.	1·45	1·37

Mean for the two days — 1·41.

TABLE VII

*1940 Experiment. Respiration (mg. CO_2 per hr. per gm. dry matter) of
Roots of the Non-nodulated Plants at an Early Fruiting Stage*

Jar.		Sept 3.	Sept. 4. 1st period.	Sept. 4. 2nd period.	Sept. 5. 1st period.	Sept. 5. 2nd period.
5A	. .	1·31	1·96	1·90	1·54	1·28
6A	. .	1·59	1·74	(lost)	1·39	1·34
7A	. .	1·67	1·52	1·69	1·42	1·64
8A	. .	1·48	1·04	1·28	1·07	1·18
Mean	.	1·52	1·61		1·36	

Mean for the three days = 1·50.

TABLE VIII

*1940 Experiment. Respiration (mg. CO_2 per hr. per gm. dry matter) of
Nodules of Inoculated Plants at an Early Fruiting Stage.*

Jar.			Sept. 3.	Sept. 4.	Sept. 5.
5	.	.	5·63	5·09	4·54
6	.	.	3·62	6·80	2·77
7	.	.	5·27	5·89	5·42
8	.	.	5·74	4·34	4·51
Mean		.	5·07	5·53	4·31

Mean for the three days – 4·97.

Note. Each figure for Sept. 4 and 5 is the average of two successive periods (see text), the actual amount (mg.) of carbon dioxide evolved from the roots and nodules together during the individual periods being as follows:—Sept. 4, jar 5, 29·86 and 28·05; jar 6, 28·62 and 27·71; jar 7, 36·08 and 35·75; jar 8, 24·44 and 25·33; Sept. 5, jar 5, 26·29 and 24·61; jar 6, 16·23 and 13·20; jar 7, 32·56 and 32·22; jar 8, 24·40 and 23·28.

appeared in respect of the dry weights and nitrogen contents of the individual jars of nodulated plants (Table I). The measurements made on this occasion concerned only roots and nodules, and the results are to be found in Tables VII and VIII. Observations extended over three days, and on the second and third days two successive determinations each of three hours' duration were made on each jar of plants. It will be observed from Table VII that the degree of agreement between consecutive periods was variable in the case of the non-nodulated plants, and actually the differences, reckoned for each pair of determinations as a percentage of the figure for the first period, were as

follows (starting with the observations on jar 5A on Sept. 4): 3, 11, 23, 17, 4, 15, 10. In some cases the changes were gains, in others losses. While the occurrence in some cases of such considerable variation of results was un-expected, that actual fluctuation in respiration rather than unsuspected experi-mental error was responsible is suggested by the fact that the agreement in the case of the nodulated plants was much closer, although the procedure was identical for both groups of plants. The footnote to Table VIII gives the amount of carbon dioxide liberated from the roots and nodules of each jar during the consecutive periods. Calculation shows that the differences in seven out of eight pairs of determinations did not exceed 7 per cent.

The average figure for nodule respiration over the three days is rather more than three times that for root respiration. Both values are lower than those obtained at the earlier stage. Attention is drawn to the variation in respect of the results for jar 6 on different days (Table VIII).

TABLE IX

Comparison of Respiration of Roots and Nodules of the Inoculated Plants

		Respiration (mg. CO_2/hr./ gm. dry wt.).	Dry wt. of 5 plants.	Respiration of 5 plants (mg. CO_2 per hr.).	Percentage of total respira-tion of roots and nodules.
1939 Expt. flowering stage	Roots	1·56	1·85	2·89	47
	Nodules	5·02	0·65	3·27	53
1940 Expt. pre-flower-ing stage	Roots	2·38	1·10	2·62	43
	Nodules	6·52	0·54	3·52	57
1940 Expt. early fruit-ing stage	Roots	1·49	1·78	2·65	30
	Nodules	4·97	1·26	6·26	70

The foregoing results of the determinations of respiration will now be considered on the absolute basis. Since in two series of determinations out of the three conducted roots and nodules only were considered, the absolute respiration of these organs will first be compared. Table IX presents the relevant figures. In the right-hand column of the table the respective respira-tion of roots and nodules is expressed as a percentage of the total respiration of roots and nodules together. In making these calculations the values for respiration per unit dry weight of root tissue obtained in the observations on the non-nodulated plants are of course employed. It will be observed that in the 1939 experiment, at the flowering stage, the total respiration of the nodules was slightly in excess of that of the root system. In 1940, at the pre-flowering stage, a rather similar state of affairs obtained, but by the time that the early fruiting stage had been reached the total evolution of carbon dioxide from the nodules was more than twice that from the root system. This was the

result of an increase in the weight of nodules relative to the roots, combined with a rather more marked fall in the respiration of the roots.

The observations made at the pre-flowering stage in the 1940 experiment included some on the respiration of tops, so that the respiration of all parts of the plants can be accounted for at this stage. Table X shows how the evolution of carbon dioxide from tops, roots, and nodules compared. At this stage the respiration of the nodules amounted to one quarter of the respiration of the plant as a whole inclusive of nodules.

DISCUSSION

In the first place it is of interest to compare the figures obtained in this work for the respiration of leguminous roots and nodules, expressed on the basis of unit dry weight, with the corresponding results of Allison and his collaborators. Though they paid most attention to the absorption of oxygen, the evolution of carbon dioxide was frequently measured as well, and since in their tables the respiratory quotients are given for such experiments, it is possible to calculate the evolution of carbon dioxide, thus facilitating comparison with the results obtained in the present work. Only those observations of Allison et al. relating to roots and nodules of soya bean respiring in air are considered.

In expt. 38 (Allison, Ludwig, Minor, and Hoover, 1940, Table I), on small roots, an oxygen consumption of 1·92 cu. mm. per hr. per mg. dry matter is reported and an R.Q. of 0·87, indicating an evolution of 3·30 mg. carbon dioxide per hr. per gm. dry matter. In expts. 21 and 22 on similar roots, assuming again an R.Q. of 0·87, an evolution of 4·30 and 4·74 mg. carbon dioxide is indicated. Expt. 38 included a determination on large roots of oxygen as 0·57 cu. mm. with an R.Q. of 1·33, thus indicating an evolution of 1·50 mg. carbon dioxide. In Table I of the communication by Allison, Ludwig, Hoover, and Minor (1940) five values for the oxygen intake of small soya bean nodules are given, ranging from 1·39–2·05 cu. mm. The stated values for the R.Q.s indicate that in terms of carbon dioxide the range becomes 3·38–7·25 mg. In expt. 37 on large nodules the oxygen was 0·76 cu. mm. and the R.Q. 2·00, indicating an output of 3·00 mg. carbon dioxide/hr./gm. dry matter.

These data are still not directly comparable with those of the present paper since the working temperature in the experiments of Allison et al. was 28° C., some 7–9° higher than in the present investigation. Assuming however that the Q_{10} for the respiration of these organs is 2, and taking the temperature difference as 8° C., the following figures are obtained when the results of Allison et al. are adjusted to the lower temperature. All data relate to mg. carbon dioxide/hr./gm. dry matter.

Allison et al.		Bond.
Small roots . . 1·98–2·84		1·49–2·38 (averages for roots of all sizes)
Large roots . . 0·46		
Small nodules . . 2·02–4·36		4·97–6·52 (averages for nodules of all sizes)
Large nodules . . 16·9		

One further point to be noted is that in all these experiments of Allison et al. glucose was supplied to the excised organs during the determinations. This was found in tests with other legumes to stimulate the root respiration to an average extent of 40 per cent., though the effect on nodules was much less. It does not seem possible to judge which values (i.e. those obtained in the presence or those in the absence of glucose) are to be regarded as approximating most nearly to the rates of respiration prevailing prior to excision.

It is perhaps possible to conclude that the figures for the respiration of soya bean roots obtained in the author's investigations are of the same order or slightly higher than those reported by Allison et al.[1] There is, of course, this difference between the experiments, that whereas the observations of the latter authors were actually made on the roots of nodulated plants, those in the present case were made on the roots of non-nodulated plants; as stated, however, the indications are that the respiration of the roots of the author's nodulated plants was similar to that of the non-nodulated groups. The list of figures given above shows that the respiration of nodules in the experiments of Allison et al., in terms of carbon dioxide evolved, was rather higher than that of the roots. The nodules examined by the present author appear to have been characterized by a considerably more intense respiration, and on the basis of unit dry weight about three times as much carbon dioxide was evolved from the nodules as from the roots. It is possible to mention certain factors which may have contributed to the discrepancies which are obviously present in the results of the two investigations. In the first place the author's observations were made on nodules still attached to the plant, while Allison et al. used detached nodules. That this contributed materially to the difference in results is made rather doubtful by the fact that Dr. G. F. Asprey, in unpublished experiments by a method already mentioned (p. 324), in which detached nodules from plants similar to those forming the subject of the present author's experiments were used, has obtained equally high values for nodule respiration. A further point is that Allison et al. made their observations on roots and nodules from field-grown plants, which would be exposed to conditions appreciably different from those under which the author's plants were grown. It is possible that the latter conditions were unusually favourable to nodule activity, and it has in fact already been noted that the dry weight of nodules relative to that of the plant as a whole was supranormal. The probable use of different varieties of soya bean in the two investigations does not appear likely to have had much effect, but more importance may perhaps be attached to the probability that the plants were infected with different strains of the nodule organism. The possibility exists that different strains vary in their respiration as well as in the ability to fix nitrogen. It will, however, need

[1] The review by Thomas (1930) indicates that investigations by previous workers on the roots of various plants in different rooting media and conditions have yielded very divergent values for respiration, ranging from 0·15 to 3·00 mg. carbon dioxide per hr. per gm. dry matter.

further experiments to elucidate the differences in results for the respiration of the nodules.

Before leaving the consideration of respiration per unit dry weight of roots and nodules, reference may again be made to the finding that there was a fall in the respiration of both organs between the first and second series of determinations in the 1940 experiment. This is in keeping with the observations of Kidd, West, and Briggs (1921) in respect of various organs of the sunflower plant. In an earlier paper (Bond, 1935) it was reported that in another respect, namely, fixation of nitrogen, there was an average decrease in activity of unit dry weight of soya bean nodule tissue as the plants aged. The opportunity is taken of referring to recent criticism by Raju (1939) who states that in this connexion the present author assumed that the amount of nitrogen fixed is in all cases dependent on the weight of nodules formed. So far as the author is aware, no such assumption was made or implied; his conclusions concerning decreased fixation of nitrogen were based directly on the experimental data.

TABLE X

Comparison of Respiration of Tops, Roots, and Nodules of the Inoculated Plants at the Pre-flowering Stage (1940 Experiment)

	Respiration (mg. CO_2 /hr./ gm. dry wt.).	Dry wt. of 5 plants.	Respiration of 5 plants (mg. CO_2 per hr.).	Percentage of total respiration of whole plant.
Tops . . .	1·41	5·67	8·01	56·6
Roots . . .	2·38	1·10	2·62	18·5
Nodules . . .	6·52	0·54	3·52	24·9

For the remainder of the discussion attention will be paid chiefly to the respiration of the nodules and of other parts of the plant when the absolute method of expression is used (Tables IX and X). It has already been noted that, according to the data presented in Table X, at a pre-flowering stage the total respiration of the nodules present on a plant amounted to one quarter of the respiration of the plant as a whole, inclusive of nodules. At all three stages at which observations were made, the total respiration of the nodules present on a plant was in excess, to a varying extent, of that of the roots alone of the plant (Table IX). So far as the evolution of carbon dioxide is a satisfactory basis for comparison, the above conclusions indicate how the utilization of carbohydrates in the respiratory processes of the nodules compared in amount with that of the other parts of the plant.

It is also possible to draw certain conclusions, in respect of the period in development intervening between the first and second series of observations in the 1940 experiment, on the relation between the amount of carbohydrate consumed by the nodule tissues, as judged by the evolution of carbon dioxide, and the total quantity of carbohydrates synthesized by the host plant during the period. For several reasons any calculations along these lines can only be of

an approximate nature, chiefly because no close control of conditions in the greenhouse was attempted, so that rates of respiration by nodular and plant tissues would often be appreciably different from those prevailing during the actual determinations. In respect of temperature, possibly the most important variable, the range selected as standard for the determinations of respiration was somewhat above the mean temperature for day and night in the greenhouse.

The total amount of carbohydrate synthesized during the period of 43 days intervening between the first and second stages is indicated approximately by the increase in dry weight of the plants, plus the amount utilized in the respiration of the various parts of the plants. The average increase in dry weight for five nodulated plants was 6·4 gm. (Table I), to which is to be added 2·9 gm., the estimated dry weight of leaves falling from the plants during the period. This gives a total of 9·3 gm., which is perhaps best reckoned as hexosan, corresponding to 10·3 gm. hexose. The absence of a figure for top respiration at the second stage introduces an element of uncertainty into the calculation of the total respiration of the plants during the period. It may, however, reasonably be assumed that a diminution in intensity of top respiration per gm. dry weight of the same order as that detected in roots and nodules (say 30 per cent.) occurred during the period. In this case a value of 1 mg. carbon dioxide/hr./gm. dry weight is indicated for tops at the second harvest, and on this basis the following figures are arrived at:

Respiration of 5 complete plants on, say, July 23 (Table X) $=$ 14·2 ⎫ mg. carbon
      ,,   ,,   ,,   ,,   Sept. 4   = 19·5 ⎭ dioxide per hr.

Assuming that the average level of respiration during the interval of 43 days is indicated by the arithmetic mean of these initial and final values, a total production of 17·4 gm. carbon dioxide, equivalent to 11·9 gm. hexose sugar, is signified for the period. Thus total photosynthesis = 10·3 + 11·9 = 22·2 gm. hexose.

A similar calculation can be made of the respiration of the nodules alone, using data presented in Table IX:

Respiration of the nodules of 5 plants on July 23 = 3·52 ⎫ mg. carbon dioxide per hr.
      ,,   ,,   ,,   ,,   Sept. 4 = 6·26 ⎭

The arithmetic mean being 4·9 mg., a production of 5·1 gm. carbon dioxide, corresponding to 3·5 gm. hexose, is indicated for the period of 43 days.

The final conclusion is that out of 22·2 gm. carbohydrate synthesized by five plants during the period, 3·5 gm., or 16 per cent., was consumed in the respiration of the nodules. In view of the various approximations and assumptions involved in arriving at these figures, nothing more is claimed for them than that they reflect the order of carbohydrate consumption within the nodules relative to the rest of the plant. In the calculations the rate of evolution of carbon dioxide has been taken as the basis of estimation of carbohydrate

consumption. Attention has already been drawn to the important fact that the two are not necessarily proportional, and the various considerations mentioned on p. 316 suggest that the consumption of carbohydrates within the nodules may actually have been higher than is indicated above. As has been mentioned already (p. 314), Allison (1935) concluded provisionally that the data then available indicated a consumption by the nodule bacteria of not more than 3–6 per cent. of the total carbohydrate synthesized. The level of carbohydrate consumption within the nodules of the particular plants examined by the present author appears to have been higher than was anticipated by Allison, probably chiefly as a result of the very abundant nodulation and of the high rate of respiration prevailing in the nodules. It is perhaps worth noting that the present conclusions are in respect of the second half of development of the plants, when katabolic processes generally would be more intense relative to those of the anabolic type than in earlier stages of development.

The data secured in this investigation make it possible to attempt to calculate the consumption of carbohydrate occurring in the nodules by a second method, that of Christiansen-Weniger (see Introduction), and it is of interest to compare the figures arrived at by this method with those based on the respiration measurements. It has been pointed out that the increase in average dry weight of the nodulated plants between the first and second harvests in 1940 amounted to 6·36 gm. for five plants, the corresponding figure for the non-nodulated plants being 9·56 gm. (Table I; the error due to loss of leaves was similar for both types of plant). According to the argument of Christiansen-Weniger the difference between these figures (i.e. 3·2 gm.) is a measure of the consumption of organic matter within the nodules of five plants. This figure is quite close to that based on the respiration measurements (3·5 gm.), though in view of the approximate nature of the latter no great significance can be attached to the agreement between the two figures. As already pointed out, Allison (1935) has discussed several sources of error in the method of Christiansen-Weniger, and he considered that most of these would tend to make the apparent utilization of material within the nodules greater than in actuality.

So far in this account no differentiation between the physiological activity of different parts of the nodule has been attempted. Plant tissues are, of course, present as well as the bacteria, and an effort has been made to obtain some idea of the respective contributions of the bacteria and of the plant tissues of the nodule towards the total respiration of the nodule as a whole. As a first step a determination was made of the relative dry weights of the central infected (the so-called bacterial) tissue, and of the peripheral uninfected tissues of the nodule. This was done by direct separation of the two regions of the nodule. Medium and large nodules were selected for the purpose from soya bean plants growing under the same conditions as those used in the observations on respiration. The following figures show the percentage of the total dry

weight that was due to the bacterial tissue and to the peripheral uninfected tissue respectively:

	Bacterial.	Peripheral.
Sample 1 (20 nodules, diam. 5–9 mm.) . . .	56	44
„ 2 („ „ „ 4–8 mm.) . . .	53	47
„ 3 („ „ „ 5–7 mm.) . . .	48	52

The average proportion for the three samples is 52 : 48. Examination of the total dry weights of the samples confirmed that the average size of nodules decreased slightly from samples 1–3, and it is perhaps no accident that the highest figure for the proportion of bacterial tissue was obtained for the sample which included the biggest nodules, which is what would be expected.

In its morphological composition the peripheral uninfected region of the nodule is very similar to the root, including as it does meristematic, parenchymatous, mechanical, and conducting tissues, and it seems most likely that the respiration of this portion of the nodule will be of the same order of intensity as that of roots, on the basis of unit dry matter. If we assume this, and knowing that the bacterial and peripheral tissues contribute almost equally to the dry weight of the nodule, a simple calculation shows that for example at the first stage in the 1940 experiment, where values of 2·38 for root, and 6·52[1] mg. carbon dioxide/hr./gm. dry matter for nodule respiration were obtained, the respiration of the bacterial tissue must have been at the rate of 10·6 mg. carbon dioxide/hr./gm. dry matter. Of the total respiration of 6·52 mg. carbon dioxide/hr./gm. dry matter of nodule tissue as a whole, the bacterial tissue constituent was responsible for 6·52—1·19 = 5·33 mg. or rather more than three-quarters of the total. A similar calculation for the second stage in the same experiment indicates that the bacterial tissue was then responsible for rather more than four-fifths of the total respiration of the nodule. Since the bacterial tissue also includes some living plant material, the proportion of the total nodule respiration that was due to the actual bacteria would be somewhat lower than these figures suggest, while on the other hand the respiration per unit dry weight of bacteria alone may have been still higher than 10·6, to revert to the example mentioned above.

As a final point the quantitative relation between the utilization of carbohydrates within the nodules and the process of fixation of nitrogen may be considered. The increase in the nitrogen content of five average nodulated plants between the first and second harvests of the 1940 experiment amounted to 159 mg. (Table I), which, after the addition of 30 mg., the estimated nitrogen content of leaves which fell from the plants, represents the fixation of nitrogen during the interval. Therefore the fixation of 189 mg. nitrogen was attended by a consumption of 3·5 gm. carbohydrate within the nodules (p. 332). Thus on the basis of the methods adopted in this work it appears that for each mg.

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[1] It is realized that a certain (probably small) error is introduced by the use of this figure (which is an average for all the nodules present on the plants) in a calculation concerning only the medium and larger nodules.

of nitrogen fixed there was a consumption within the nodules of some 19 mg. of carbohydrate, of which probably about 15 mg. was utilized by the bacteria alone. Though it is not claimed that these figures do anything else than indicate the approximate level of the relation between the two processes, they do perhaps represent a useful addition to the very scanty data of this type that are already available. Christiansen-Weniger, from his experiments referred to in the introduction, concluded that for each mg. of nitrogen fixed there was a consumption of 5·6 mg. dry matter in the nodules of bean (*Vicia Faba*) and 7·2 mg. in the case of lucerne. Corresponding figures reported by Allam (1931) in two experiments were 26·0 and 12·6 mg. The indication of these investigations is that the energy requirements of the process of nitrogen fixation in legume nodules is small, a conclusion which appears to be supported by the results of the present study, having regard to the fact that the above consumption of carbohydrate (15 mg. per mg. of nitrogen fixed) covers the katabolic activities as a whole of the bacteria, so far as they are attended by evolution of carbon dioxide.

SUMMARY

Determinations have been made of the rates of evolution of respiratory carbon dioxide from the nodules, roots, and tops of intact leguminous plants (soya bean) growing in water (or solution) culture. The values for root respiration were established by means of observations on non-nodulated plants receiving nitrate-nitrogen. The determinations were made at three different stages in the development of the plants.

Though considerable variation in results was experienced, on each occasion the respiration per unit dry weight of tissue was in the nodules approximately three times that of the roots. On an absolute basis the combined respiration of all the nodules on given plants was greater, though to a varying extent, than that of the roots. At a stage shortly prior to flowering, the nodular respiration amounted to 25 per cent. of that of the plant as a whole, the roots accounting for 18 and the top for 57 per cent. It is presumed that these figures indicate also the respective consumption of carbohydrate for respiratory purposes by the various parts of the plant.

During a period extending from shortly before flowering to the stage of early fruit formation, the consumption of carbohydrate within the nodules of a plant amounted to 16 per cent. of the total carbohydrate synthesized by the host plant during the period. For each mg. of nitrogen fixed during this period there appears to have been a consumption of 19 mg. of carbohydrate within the nodules. These figures should be regarded as approximations only.

Evidence is advanced which suggests that the bacteria probably accounted for something like three-quarters of the respiration of the nodule as a whole.

The accuracy of these conclusions is dependent especially upon the extent

to which the rate of formation of carbon dioxide is a satisfactory basis for the comparison of the utilization of carbohydrate in the katabolism of different biological material. Other sources of error are discussed in the text.

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ADDENDUM

P. W. Wilson, in his recent monograph ('The Biochemistry of Symbiotic Nitrogen Fixation', 1940), includes some data on the oxygen-intake in respiration of nodules, roots, and tops of several leguminous species. He also presents a critical discussion of the method of calculating the energy requirements of fixation employed by Allam (see above) and also by W. B. Andrews in a recent paper ('Effect of ammonium sulphate on the response of soybeans to lime and artificial innoculation and the energy requirement of soybean nodule bacteria.' Jour. Amer. Soc. Agron., 29, 681–9, 1937).

The Structure of *Anthoceros laevis* in relation to its Water Supply

BY

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With seven Figures in the Text

*A*NTHOCEROS LAEVIS is a very common member of the Anthocerotales, growing in areas of abundant moisture, e.g. moist soil in fields, sides of ditches and streams, or even submerged. The plants are monoecious with the sex organs appearing on the dorsal surface. The prostrate thallus varies from 5 to 15 mm. in width, the edges being somewhat lobed, and the plants branching dichotomously. The growth frequently becomes crowded, with the result that the edges overlap and the branches tend to become sub-erect. The sporophyte is erect, attaining under favourable conditions a length of 15 to 25 mm. The early stages of development of the sporophyte are accompanied by some growth of the adjacent gametophytic tissue, resulting in the formation of the involucre, which grows with the sporophyte for a time, reaching a maximum height of about 5 mm. With the progressive growth of the sporophyte this involucre remains as a collar around its base (Fig. 1).

The mode of absorption and conduction of water by this rapidly growing sporophyte appeared to be a problem worthy of investigation, which involved an examination of both the gametophytic and sporophytic anatomy.

Gametophyte.

The essential features of the anatomy of the gametophyte are well known. A somewhat regular layer of cells occur on the dorsal surface, followed by three to six layers of large irregular parenchymatous cells with air spaces between them, and a ventral limiting layer of cells from which numerous smooth walled rhizoids develop. There are no scales on the ventral surface. Most of the cells of the thallus, especially those near the dorsal surface, contain a single large chloroplast. As in the case of other species of Anthoceros, many intercellular cavities occur, filled with mucilage, opening to the ventral surface by narrow slits, and occupied by the familiar Nostoc colonies. The presence of the mucilage was proved by the application of 0·5 per cent. solutions of methylene blue and toluidin blue respectively to sections of the thallus, resulting in a positive reaction by practically all the cells, including the rhizoids. The region of the Nostoc colony showed the most abundant occurrence of mucilage.

A preliminary investigation of the path taken by liquid external to the plant was made by washing suitable specimens, freeing them from surplus water by means of blotting-paper, and then placing them on prostrate wads of filter-paper in shallow glass dishes containing 0·5 per cent. solutions of 'vital' stains, such as vital red, methylene blue, neutral red, trypan red, pyrrol blue, and diamin black respectively, the last-named stain having been dissolved in a 1·0 per cent. solution of sodium chloride. The plants were in contact on their ventral surface with the saturated filter-paper, but were not immersed in the liquid, and each dish was covered by an inverted glass pneumatic trough (Fig. 2) in order to maintain a moist atmosphere. After a few minutes, examination under the binocular microscope showed that liquid had passed over the margins of the thallus on to the dorsal surface, where it quickly spread. Later, sections showed that the stain had entered the cells of the dorsal and ventral limiting layers and also the rhizoids, while invariably the Nostoc colony and the surrounding cells showed the presence of the stain before any coloration was apparent in the remainder of the internal tissue.

FIG. 1. Thallus of *A. laevis* bearing sporophyte. *sp.*, sporophyte; *in.*, involucre; *th.*, thallus; *rh.*, rhizoids.

Further series of experiments were carried out in which the stains were replaced respectively by (*a*) 0·1 per cent. solution of potassium nitrate (sections of the plant so treated being mounted in a drop of diphenylamine in concentrated sulphuric acid and examined at once for the blue colour characteristic of the nitrate), and (*b*) 0·1 per cent. solution of ferric chloride (sections of these plants being mounted in a drop of ammonium sulphide solution, a black deposit of ferric sulphide denoting the presence of iron). The results of these experiments again showed that the liquids had passed over the margins of the thalli on to the dorsal surface and penetrated into the cells of the dorsal and ventral limiting layers, and into the rhizoids. It is, therefore, evident that in *A. laevis* absorption of water occurs over the whole surface and is not located to any particular region.

All of the previous experiments were then repeated with fertile thalli, and, in every case, examination within a few minutes of exposing the plant to the test liquid showed that the archegonia and antheridia were deeply saturated, although within that brief period the internal tissue ventral to the sex organs showed no trace of the presence of the stain, potassium nitrate, or ferric chloride respectively. Hence it appears probable that in nature, as in the case of Pellia (Clee, 1939) the sexual organs receive ample

water conducted in the form of capillary films over the external surface of the thallus. The spread of these external films of liquid probably facilitates fertilization by carrying the swimming sperms along with them, and so aiding them in reaching the oospheres, especially since the rapid entry of the test liquid into the neck canal of the archegonia suggests its rapid absorption by the mucilage there.

The sporophyte.

The sporophyte of *Anthoceros laevis* is like an elongated spindle, with a
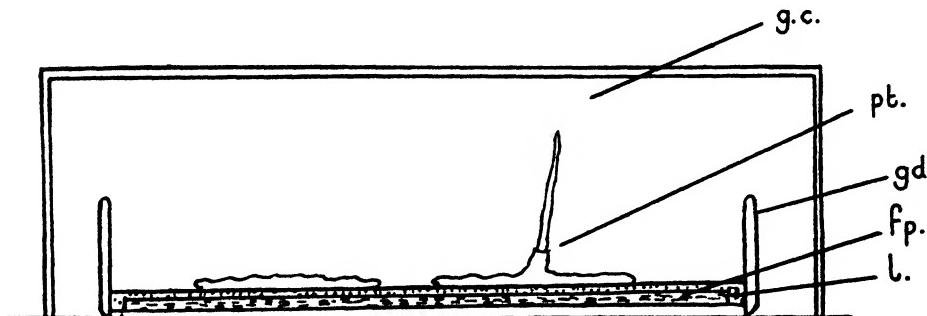
FIG. 2. Diagram of apparatus used. *g.c.*, glass chamber; *pt.*, plant; *gd.*, glass dish; *fp.*, filter-paper; *l.*, liquid.

large bulbous foot embedded in the gametophyte, an intercalary meristematic region, and a long green capsule. When young the sporophyte is green to the tip, but later it becomes brownish, and, as it ripens, it ruptures longitudinally by slits which extend progressively downwards. The lower part of the sporophyte is surrounded by the aforementioned involucre of gametophytic tissue which, it is suggested, gives mechanical support to 'the weak intercalary zone' (Bower, 1935). This involucre is seen on close examination to be irregularly ridged, while its base is surrounded by a shallow moat-like depression in the thallus (Fig. 5). There is no cleft or mucilage layer comparable to that found by Clee in Pellia at the base of the foot. The bulbous foot probably serves the double functions of anchorage and absorption, and the absorbing power of the basal region is increased by the papillate nature of the peripheral cells of the foot which impinge upon the gametophytic tissue (Fig. 3).

In the capsule the central columella consists of elongated thin-walled cells, which appear in transverse section as a square of 16 cells. A longitudinal section shows that the end walls of these cells are very much oblique, the cells dovetailing in a manner similar to that of fibres and tracheides (Fig. 4). The archesporium, as a single layer of cells, can be distinguished quite easily, even in the meristematic region.

Outside the archesporial tissue occur about four to six layers of thin-walled cells. These cells on the outer region near the epidermis each contain two chloroplasts. Also, scattered throughout this parenchymatous tissue are seen

empty cells which are smaller than the normal ones in transverse section, but they are somewhat elongated longitudinally (Fig. 4). These cells form longitudinal series, which appear to run independently, for no evidence of their direct contact with one another or with the epidermis was obtained.
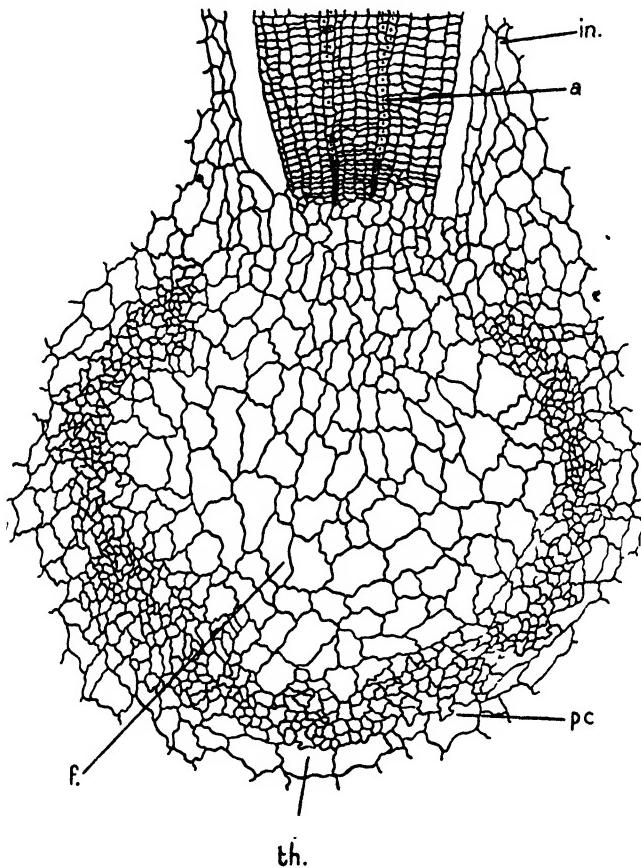
FIG. 3. Longitudinal section through the sporophyte of *A. laevis* showing meristematic and foot regions. *in.*, involucre; *a.*, archesporium; *f.*, foot; *pc.*, papillate cells; *th.*, thallus.

The epidermal cells are quite distinct from those underlying them. Their outer walls are papillate, increasing the outer surface of the sporophyte.

In the region covered by the involucre, these epidermal cells are filled with dense protoplasmic contents, but higher up in the green capsule they are empty. It has been suggested by former workers (Campbell, 1924; Smith, 1938) that the epidermal cells of *A. laevis* are strongly cutinized, but the writer, testing with scharlach red, failed to find any trace of cutin. In surface view these epidermal cells are narrow and elongated with end walls which are frequently oblique. Stomata, though present, are not numerous.

Longitudinal and transverse sections of the sporophyte were treated with

methylene blue and toluidin blue respectively, to test for the presence of mucilage. The cells of the foot and columella, the scattered elongated cells of the parenchymatous region, and the papillate epidermal cells gave a positive reaction. The 'cutin', described by earlier workers, on the external wall of the epidermis appears to be really a mucilaginous layer.
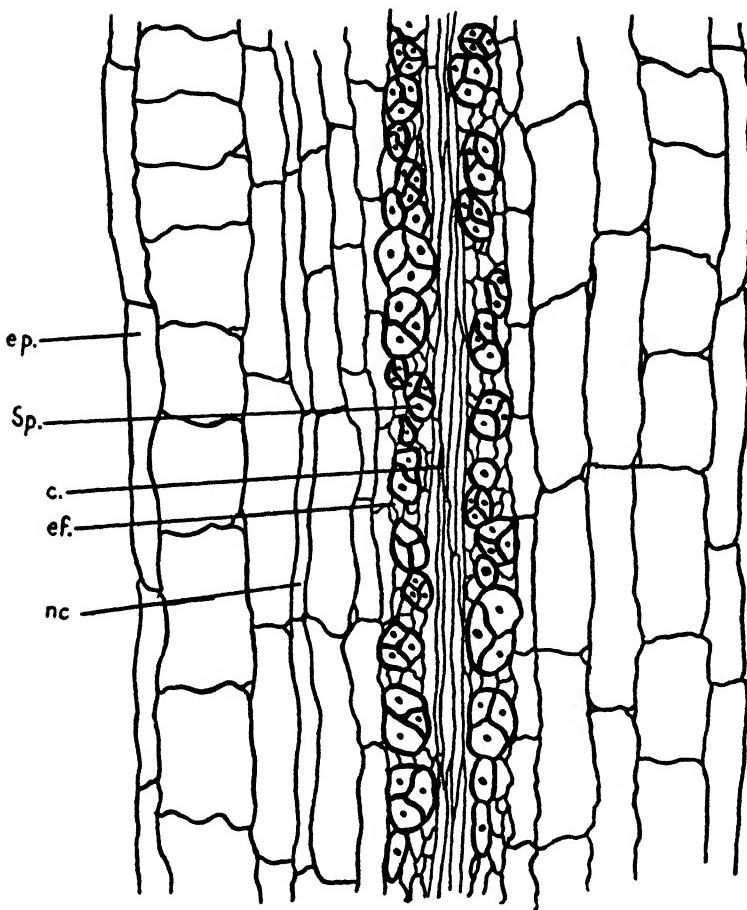
FIG. 4. Longitudinal section through the mature sporophyte. *ep.*, epidermis; *sp.*, spores in tetrads; *c.*, columella; *ef.*, elaters; *nc.*, narrow elongated mucilaginous cell.

Experiments with vital stains, similar to those recorded above for the vegetative and fertile gametophytes, were now carried out with thalli bearing developing sporophytes, in order to trace the path of external liquid (Fig. 2). Examination every few minutes under the binocular microscope showed that the liquids passed quickly over the edges of the thallus on to the dorsal surface and soon filled the moat-like depression around the base of the involucre. From this they passed up externally over the involucre, the ridges of the latter

aiding the rise of the capillary films. Later, careful examination of sections showed the stain passing down into the space between the involucre and the sporophyte. Some of the coloration was also seen in the cells of the involucre. Other sections showed the stain passing from the depression at the base of the
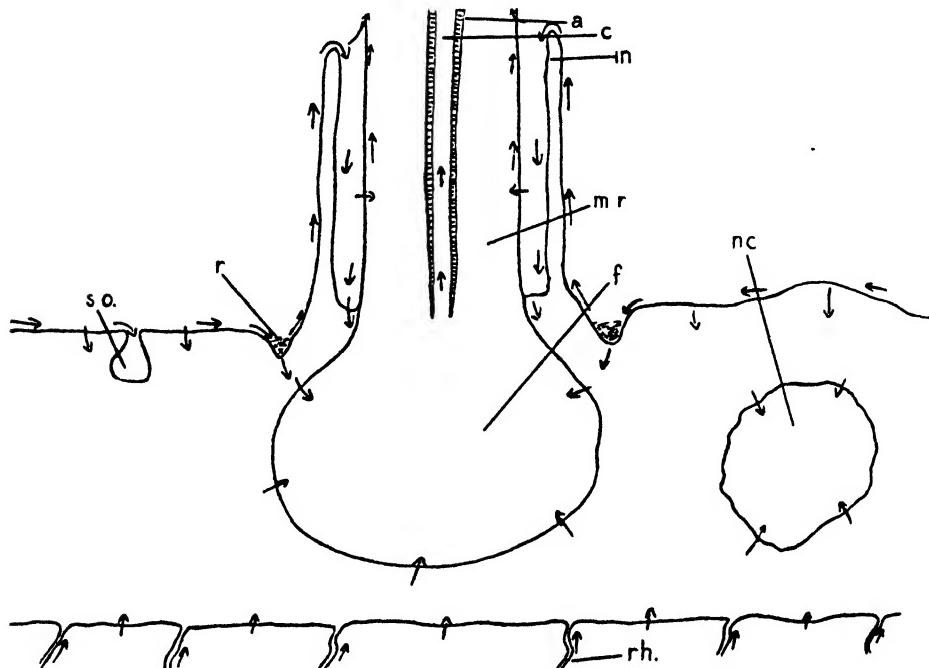
FIG. 5. Diagram to show the path taken by water in reaching the sporophyte. Arrows indicate the path taken. *a.*, archesporium; *c.*, columella; *in.*, involucre; *m.r.*, meristematic region; *f.*, foot; *nc.*, Nostoc colony; *s.o.*, sex organs; *r.*, reservoir; *rh.*, rhizoids.

involucre into the cells of the gametophyte towards the foot of the sporophyte. The moat-like depression seems therefore to act as a reservoir from which water can pass in two directions, viz. externally up over the involucre to the outer surface of the sporophyte, and internally through the cells of the gameto-phyte into the foot of the sporophyte. The internal supply to the foot of the sporophyte is also apparently augmented by liquid entering through the ventral surface of the prostrate thallus and passing in towards the mucila-ginous cells of the foot, for, after a time, the stains could be plainly seen in this region (Fig. 5).

Although the vital stains could be clearly observed in the gametophytic tissue surrounding the sporophyte, and could therefore be used as an indica-tion of the path of conduction to the sporophyte, they were never visible within the tissues of the latter, and so were useless for purposes of determination of the path of travel of water within the diploid generation. (This seems to indicate an interesting physiological difference between the two generations.)

For this purpose potassium nitrate and ferric chloride respectively were successfully used. Sections of plants exposed to these liquids and treated with the appropriate reagents showed that the solutions penetrated into the thallus and passed very quickly through it to the dorsal surface and so reached the cells of the foot of the sporophyte before any other part of this generation showed their presence. They also passed over the edges to the dorsal surface of the thallus, and could soon be detected passing up over the external surface of the involucre as well as penetrating into its cells. From the top edge of the involucre some of the solutions passed immediately into the empty mucilaginous epidermal cells of the sporophyte at that level, while the remainder passed down the cleft towards the foot. That which entered the sporophyte passed vertically upwards in the epidermal cells and obliquely upwards in the parenchymatous region, reaching the elongated mucilaginous cells of this region, and also the columella, and passing up in these. Although the solutions of salts were continuously passing in through the prostrate gametophyte to the foot of the sporophyte and from there to the columella, their level was always higher in the epidermal cells than in the cells of the columella. In some transverse sections through the capsule it was seen that even the spores and elaters had become saturated before the columella showed any signs of the presence of the solutes. Also longitudinal sections showed that the reaction for the presence of the solute was frequently given by isolated patches of the columella tissue, suggesting that its presence there was due largely to penetration from the epidermal cells through the parenchymatous and spore-bearing regions, rather than to a direct rise in the columella after absorption by the foot from the gametophyte. However, such a direct rise does take place, though comparatively slowly.

The times taken by the solution of potassium nitrate and ferric chloride respectively to travel up the sporophyte were determined, and typical results are shown in Tables I and II.

It is clear from these figures that water can reach the tip of the sporophyte of *A. laevis* within reasonable time when ample supplies are available to the gametophyte.

An attempt was then made to determine the speed at which liquid rises to the tip of the sporophyte when the supply through the internal tissue of the gameto-

TABLE I

Plant.	Length (cm.) of sporophyte.	Time (min.)	Height (cm.) of rise of potassium nitrate.
1	1·5	20	0·2 (by internal conduction)
2	0·7	30	0·5 (by internal conduction)
3	1·6	45	1·2 (by internal and external conduction)
4	1·4	60	1·4 (tip)
5	2·0	80	2·0 (tip)

TABLE II

Plant.	Length (cm.) of sporophyte.	Time (min.)	Height (cm.) of rise of ferric chloride.
1	0·8	15	0·4 (by internal conduction)
2	1·2	30	0·8 (tip)
3	1·2	40	1·2 (tip)
4	1·7	60	1·7 (tip)
5	2·3	75	2·3 (tip)

phyte is eliminated. As much as possible of the prostrate thallus was cut away, leaving the involucres surrounding the lower region of the sporophytes and the tissue underlying the foot intact. Vaseline was then smeared over the bases of the plants and very nearly to the top of the involucres, after which the plants were dipped into melted paraffin wax up to the level of the top of the vaselined area. The wax was then allowed to cool by immersing the whole in cold water. The entry of the liquid through the underside of the gametophyte to the base of the foot of the sporophyte was thus prevented. A number of short lengths of glass tubing were prepared, each of such an internal diameter as to accommodate these isolated sporophytes without damage. Each was fixed to a glass slide for support as shown in Fig. 6. The tubes were filled with potassium nitrate solution and the plants, supported by glass wool, were placed in them, so that the level of the liquid was just up to the top of the waxed area. The whole was then placed under a glass chamber to maintain a moist atmosphere. It is obvious that the only liquid available to the sporophyte was that which passed over the top of the involucre.

The times taken by the potassium nitrate to travel up the sporophyte were determined, and typical results are shown in Table III.

TABLE III

Plant.	Length (cm.) of sporophyte.	Time (min.)	Height (cm.) of rise of potassium nitrate.
1	0·7	15	0·2 up sporophyte 0·2 down cleft
2	1·0	30	0·6 up sporophyte and to the base
3	0·9	40	0·8
4	1·5	60	1·2
5	1·8	85	1·8 (tip)

A series of experiments was carried out with ferric chloride instead of potassium nitrate which gave similar results.

A comparison between Table III and Tables I and II shows that where the water supply to the sporophyte can be regarded as entirely external—passing over the surface of the gametophyte—conduction to the tip was practically as rapid as in the cases where this external supply was augmented by absorption by the gametophyte and passage through its tissues to the base of the foot. Where in these last experiments some liquid penetrated down the cleft

between the involucre and the sporophyte into the foot region, it could be seen to rise in the sporophyte in the cells of the columella.

In order to eliminate as far as possible this latter source of supply some sporophytes were dissected from the gametophytic thalli. Lengths of about
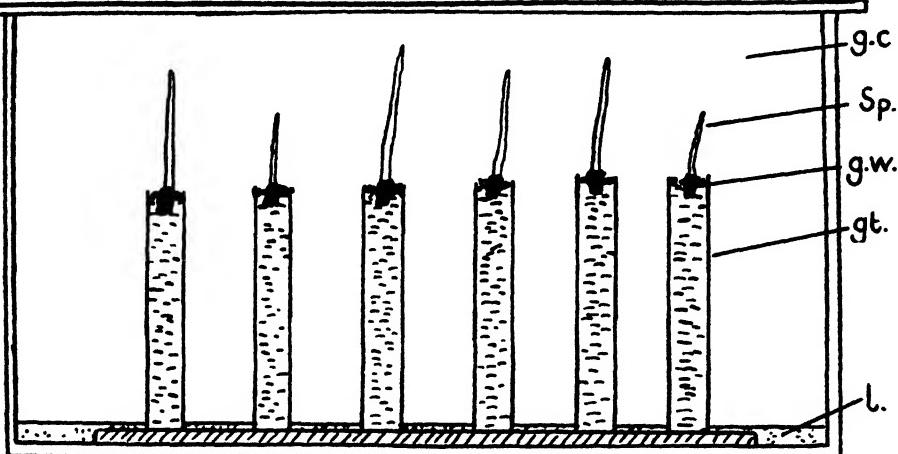
FIG. 6. Diagram to show apparatus used. *g.c.*, glass chamber; *sp.*, sporophyte; *g.w.*, glass wool; *gt.*, glass tubing; *l.*, liquid.

3 mm. of the foot regions of these sporophytes were covered by coats of vase‐ line and paraffin wax. These were then placed as described above in tubes filled with potassium nitrate and treated precisely as before. At intervals sections were cut and examined in order to ascertain the place of entry of the solute and its rate of rise in the sporophytes. The solution clearly rose quite rapidly in the epidermal cells and in the series of mucilaginous cells in the parenchymatous region. It also passed down into the foot region, and traces of it were also evident in isolated patches of the columella. Evidence was obtained that the solute in the cells of the columella was due partly to an internal ascent from the foot, and partly to transverse and oblique passage from the epidermal cells. However, the potassium nitrate had reached the tip of the capsule via the epidermal cells long before any trace of it was apparent in the upper part of the columella, except in isolated patches obviously resulting from diffusion in from the epidermis.

Typical times taken by the potassium nitrate to travel up the dissected sporophytes were as indicated in Table IV.

Experiments using ferric chloride gave similar results.

A comparison of Table IV with Table III shows that liquid reaches the tip of the sporophyte quite as rapidly by the epidermis alone as by the epider‐ mis and columella together, so that, although the latter has some conducting power, the epidermis and the mucilaginous cells of the parenchymatous region are probably responsible for the upward conduction of the greater part of the necessary supply of water.

TABLE IV

Plant.	Length (cm.) of sporophyte.	Time (min.)	Height (cm.) of rise of potassium nitrate in epidermal cells.
1	0·7	15	0·2 up and down to foot
2	0·9	35	0·7
3	1·2	60	1·2 (tip)
4	1·4	70	1·4 (tip)
5	1·7	80	1·7 (tip)

An attempt was then made to determine whether downward as well as upward passage of water is possible in the sporophyte. A number of capsules were cut away from the gametophytic thallus and their tips inserted through holes of suitable dimensions in a thin slice of cork. The cork was then placed over a glass dish with the capsules normally oriented, their cut ends being a few centimetres above the water. The latter maintained a moist atmosphere around them. A small amount of cotton-wool, saturated with potassium nitrate, was then placed on top of the cork, so that the tips of the capsules were just in contact with it (Fig. 7). At intervals sections of them were examined, and it was found that the liquid had been conducted downwards in the epidermal cells, passing from these into the internal tissue. Similar results were obtained when cotton-wool saturated with ferric chloride was used.

The epidermal cells therefore appear to play an important part in the conduction of ground water in the sporophyte of *A. laevis*. The mucilaginous nature of the walls of these cells together with their papillate form may also condition to some extent the absorption of atmospheric moisture. Water can thus reach the tip of the sporophyte of *A. laevis* without the aid of the gametophyte, and, since the former also contains quite an appreciable amount of chlorophyllaceous tissue, it appears probable that the main value of the gametophyte may lie in its power of directing supplies of water and solutes to the base of the sporophyte and in its support of the latter. If this be correct, and support and supply could be otherwise provided, the sporophyte might be expected to live, grow, and develop sporogenous tissue, even if separated from the gametophyte at an early stage. This suggestion was strengthened by the observation that when the gametophyte part of some plants kept in a cold frame died, turned black, and began to decay, the erect sporophytes continued to live, grow, and produce mature spores.

This finding is in accordance with that of Campbell (1924) for *Anthoceros fusiformis*, the sporophytes of which he was able to keep alive for three months when severed from the gametophytes, though they 'made very little growth, but ripened normal spores in a number of cases'. He suggested that the foot in this case could absorb water independently of the gametophyte, 'and so the sporophyte has become practically independent of the gametophyte and reached a condition comparable to that of the Pteridophyte after it has established its first root'.

The writer dissected sporophytes of *A. laevis* of varying sizes until they were free from the gametophytic tissue. Capillary glass tubing was cut into lengths of 2 cm. which were fastened into a bundle by means of an elastic band. The whole bundle was placed in a shallow glass dish into which Knop's
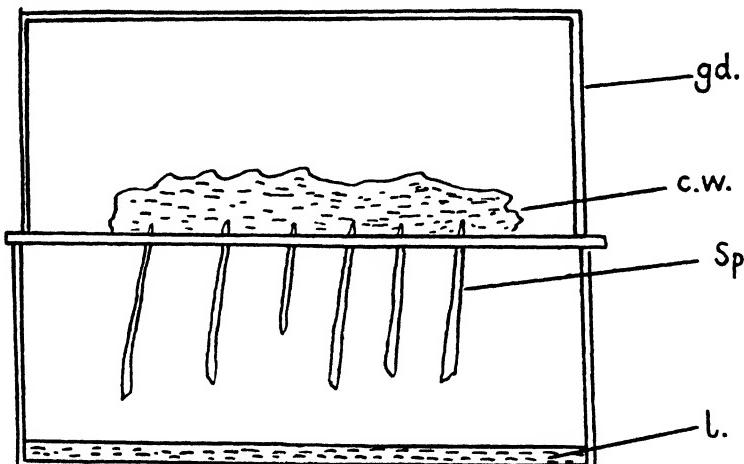
FIG. 7. Diagram of apparatus used. *gd.*, glass dish; *c.w.*, cotton-wool; *sp.*, sporophyte; *l.*, liquid.

culture solution was poured until the liquid had just risen to the tops of the tubes. The prepared sporophytes were then measured and one placed in each tube supported by glass wool. The whole was then placed under a glass chamber in order to maintain a moist atmosphere. At the same time normal plants bearing sporophytes, which had also been measured, were placed in another similar shallow dish containing Knop's culture solution, this dish being also enclosed in a glass chamber. The Knop's solution in each case was changed three times a week. At intervals all the sporophytes, both attached and unattached to gametophytes, were measured, and also examined to see when the characteristic splitting of the capsule occurred.

Though a number of the dissected sporophytes died after a few days, doubtless due to damage done to the foot region during the actual dissection from the gametophyte, the majority survived for periods of weeks, during which growth and development occurred.

The lengths of typical specimens of both these series are shown in Tables V and VI.

It is evident from these figures that though the dissected sporophytes did not grow as large and therefore did not produce as many spores as the normal ones, yet they grew and gave rise to normal mature spores when completely separated from the gametophyte at a very early stage.

This relative independence of the sporophyte of Anthoceros, together with its form, and the tendency to concentric zoning of its tissues suggest an approach to the condition in the Psilophytales, especially since some of the

Rhyniaceae were not dissimilar in size from some of the larger sporophytes of species of Anthoceros. The older conception of the parasitism of the

TABLE V.
Dissected Sporophytes

Time.	Length (cm.) of sporophyte.					
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.
Beginning of expt.	0·8	0·5	0·4	0·7	0·4	0·8
2 weeks	1·1	0·6	0·6	1·0	0·6	1·1
3 ,,	1·3	0·75	0·9	1·1	0·8	1·25
4 ,,	1·4 (spores formed)	0·8	1·0	1·25 (spores formed)	0·95	· 1·35
5 ,,	1·4 (spores formed)	0·9 (spores formed)	1·0 (spores formed)	1·4	1·1	
6 ,,	Dead	0·9 (spores formed)	Dead	1·45 (spores formed)	1·1 (spores formed)	
7 ,,	—	0·9 (spores formed)	—	1·45 (spores formed)	1·1 (spores formed)	1·6 (spores formed)
8 ,,	—	Dead		1·45 (spores formed)	1·1 (spores formed)	1·6 (spores formed)
9 ,,	—	—	—	1·45 (spores formed)	Dead	1·6 (spores formed)
10 ,,	—	—	—	Dead	—	Dead

TABLE VI
Normal Sporophytes

Time.	Length (cm.) of sporophyte.					
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.
Beginning of expt.	0·5	0·4	0·2	0·3	0·7	0·6
2 weeks	0·8	0·7	0·6	0·7	1·1	1·0
3 ,,	1·2	1·1	0·9	1·0	1·5	1·2
4 ,,	1·5	1·5	1·4	1·5	1·7	1·6
5 ,,	1·7	1·75	1·55	1·6	1·9	1·7
6 ,,	1·9	1·9	1·7	1·75	2·1	1·85
7 ,,	2·0 (spores formed)	2·0	1·85	1·9	2·2 (spores formed)	2·0
8 ,,	2·0 (spores formed)	2·1	2·0	2·1	2·2 (spores formed)	2·1 (spores formed)
9 ,,	2·0 (spores formed)	2·1 (spores formed)	2·1 (spores formed)	2·2 (spores formed)	2·2 (spores formed)	2·1 (spores formed)
10 ,,	2·0 (spores formed)	2·1 (spores formed)	2·1 (spores formed)	2·2 (spores formed)	2·2 (spores formed)	2·1 (spores formed)

sporophyte upon the gametophyte in the Bryophyta, with the support which it gives to the antithetic theory of alternation of generations, requires further

modification in the light of the present work, which, together with that of Clee (1939) and Bold (1938), tends to support the homologous view with its conception of 'a fundamental similarity in capacity for self-nutrition of the two alternating generations' (Bold). Further work is in progress on the water relations of the sporophytes of other members of the Bryophyta.

SUMMARY

1. *Anthoceros laevis* is able to absorb water over the whole of the surface of the gametophyte and sporophyte.

2. The sex organs receive practically the whole of their water supply from these external sources.

3. The water passes up over the edges of the thallus on to the dorsal surface into a shallow moat-like reservoir at the base of the involucre. Thence it passes to the sporophytes, travelling up through the mucilaginous cells of the epidermis, and also down the cleft between the involucre and sporophyte, whence it reaches the foot. After absorption by the foot it slowly rises in the columella.

4. The external rise in the epidermis is sufficiently rapid to enable the tip of the sporophyte to be well provided with water even when disconnected from the gametophyte, so long as supplies are available. Isolated sporophytes grew and produced spores, living for nine weeks after separation from the gametophytes, when placed in culture solution.

5. The water absorbed by the epidermis gradually passes inwards via mucilaginous cells to sporogenous tissue and columella.

6. Although externally conducted water and solutes can provide all the necessities for the development of the sporophyte of *A. laevis*, an appreciable amount does reach this structure internally as a result of rapid absorption by the ventral surface of the gametophyte, and the passage of the absorbed solutions through the gametophytic tissue to the base of the foot. The rate of rise in the sporophyte from this source of supply is, however, much slower than that due to the external liquid.

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Studies in Vernalisation of Cereals

VII. A Study of the Conditions of Formation and the Subsequent Growth of Dwarf Embryos of Rye

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With seven Figures in the Text

INTRODUCTION

THE following investigation was suggested by an observation in the course of experiments on vernalisation (Gregory and Purvis, 1938, p. 243). A collection was made of immature grain of spring rye in order to determine whether abnormally early drying down of the ear during development had any 'devernalising' effect. Ears of rye were harvested daily from the time of pollination, dried down at laboratory temperature, and threshed some months later. Germination tests on material so obtained showed that the ears harvested as early as five days after pollination produced viable grain. Plants grown from this dwarf grain were apparently normal in every respect.

This observation appeared to be of sufficient importance to warrant a separate investigation. In view of a theory of hybrid vigour (Ashby, 1930, 1937) relating this phenomenon to the greater embryo size in hybrids, it appeared that a study of the relative growth rates of normal and dwarf

embryos in a pure line would also have interest outside the immediate scope of this investigation. In 1938, therefore, a further collection was made so that the formation, development, and subsequent growth of normal and dwarf grain could be compared.

Historical Summary

A number of references have been found in the literature relating to the effect of premature harvest. In most of these attention is directed primarily to the agricultural aspects of the problems raised, such as the effect on germinating capacity in seed crops, the yield, vigour, and date of maturity of crops raised from immature seed, and the viability of the seeds of weed species when mown in the flowering condition, &c. The earliest of these references (Goff, 1900) mentions a breeding experiment published in 1893 (reference not given) in which a variety of tomato was grown through six generations from decidedly immature seed. This resulted in earlier maturity, a marked increase in prolificacy, and a distinct decrease in vigour. Goff records a similar experiment using three varieties of tomato. For this experiment the criterion of maturity was the colour of the fruit, no mention being made of the stage of development of the embryo when collected. No uniform effect on vigour, prolificacy, or date of maturity was evident. Only with the very immature seed was slightly earlier maturity obtained.

Harlan and Pope (1922) working with seven barley varieties discovered that 100 per cent. germination occurred if ears were removed from the plant six days after fertilization, and that 90 per cent. of the ears removed from the plant five days after fertilization gave viable grain. Earlier removal led to the death of the ovary. These authors did not study the development of the immature grain but mentioned that the grain which failed to germinate was quite without an embryo.

In a subsequent paper (1926) Harlan studied the development of the immature kernels in order to determine the possible source of nutritive materials. Some of the material harvested at an immature stage was kept moist and other material placed in the dry air of the laboratory. In some, spikelets were removed from the ears, and in others the glumes were also dissected from the kernels. As might be expected, the more drastic the treatment the smaller and more misshapen the embryos.

More recently Fleischmann (1928) by removing and air-drying the ears has investigated the germinating capacity of eight varieties of wheat, barley, and rye between fertilization and full maturity of the grain. Only the middle grains of each ear were used so that the number of days between fertilization and removal was known. Viable grain was not obtained from ears removed earlier than ten days after fertilization for wheat or sixteen days for rye. The mean weight of the dwarf seed in the latter case was 4·5 mg. compared with 10·8 mg. for grain removed at a similar stage in the present investigation.

This discrepancy may be due to varietal differences, although the variety of rye used by Fleischmann (mean normal grain weight 32·2 mg.) had almost the same size grain as that used in the present work (mean weight 32·9 mg.). Fleischmann cites Müntz's (1878) figures for water, starch, and sugar content of developing rye grain, and correlates the critical period in germinating capacity, i.e. twenty-eighth day, when percentage germination approaches 100, with the change of the sugar : starch ratio from 1 : 2 to 2 : 3.

Gill (1938) obtained viable seed from a number of weed species cut down in the flowering condition and at various subsequent stages. The species considered responded very differently to the treatment given; thus, in some cases no viable seed could be obtained unless the cut plants were dead ripe; in others a large percentage of viable seed was obtained from plants cut in the flowering condition. The experiment included plants from a number of families and the results obtained were not in any way related to the taxonomic positions of the plant concerned. Of the Gramineae, Gill found that the wild grasses *Hordeum nodosum* L. and *Bromus mollis* L., when cut in the milk-ripe stage, produced a notably high percentage of viable grain.

There is evidence, therefore, that the formation of viable seed in material harvested or cut at an immature stage is a fairly general phenomenon.

For convenience of presentation the investigation has been divided into two parts. In the first part the morphology and anatomy of dwarf and normal grain and embryos are compared, and the earliest time of successful harvest related to stages in the normal development of the grain. The results from measurements of the growth rates of plants grown from normal and dwarf grain are presented in the second part.

MATERIAL AND METHODS

The collection was made from a pure strain of winter rye, var. Petkus, grown in the open in sand culture. The date of anthesis (exsertion of anthers and stigmas), the time at which fertilization normally takes place, was noted on a label attached to each ear. These were subsequently collected at daily intervals from pollination, a number of replicates being taken for each. Some were fixed immediately and others at later intervals, the remainder being retained for germination tests and growth rate experiments. The ears, with eight to ten inches of straw attached, were cut from the plants, placed upright, and dried at room temperature until required. Of each ear only the grains from the three to four spikelets which first reached anthesis were used. Fertilization in rye occurs seven hours from pollination (Jost, 1907), so that the age of any sample was known to the nearest day.

The material for anatomical examination was fixed in 2 BE (La Cour, 1931), sectioned in paraffin wax, and stained with nuclear and cytoplasmic stains. The grain required for germination tests and growth experiments was dissected from the ears about four months from harvesting.

Comparison of dwarf and normal embryos.

Before proceeding to this comparison the gradual transition from dwarf to normal grain in material harvested at progressively later stages may be con-
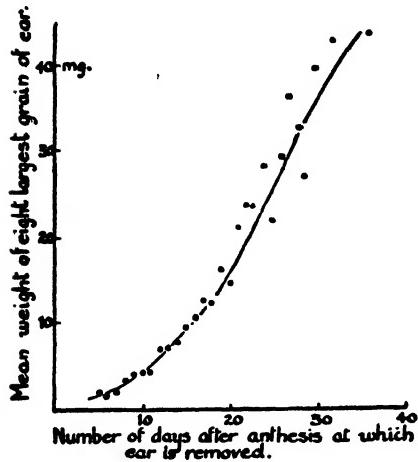
FIG. 1
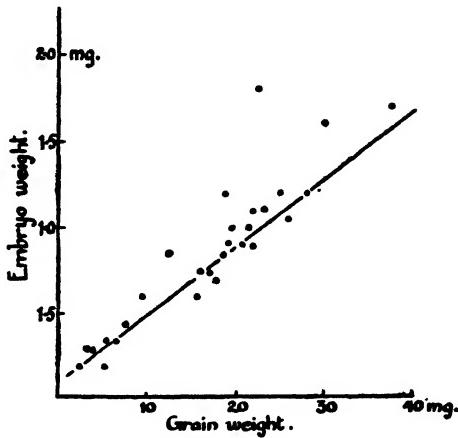
FIG. 2

FIG. 1. The relation between final grain weight and time of removal of ear from parent plant.
FIG. 2. The relation between embryo weight and grain weight in normal and dwarf grain.
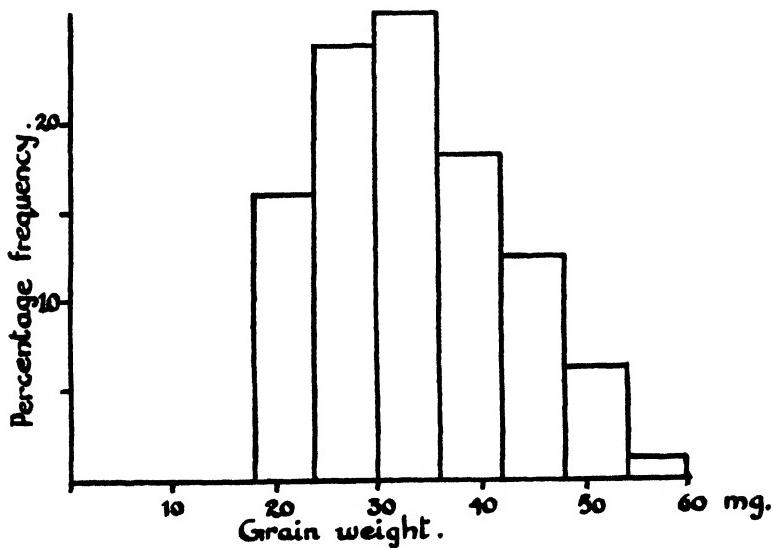
FIG. 3. Variation of grain weight in a normal harvest.

sidered. In Figs. 1–3 the following relations are shown graphically: (1) the relation between grain weight and time of removal of ear from parent plant, (2) the ratio of embryo weight to grain weight, (3) the variation of grain weight in a normal harvest. With regard to the last, it may be remarked that the

results recorded in Fig. 3 were obtained from material threshed by rolling the ears between the hands and blowing away the chaff. If, instead the grain is individually dissected from the spikelets, the much smaller grains otherwise lost with the chaff are secured.
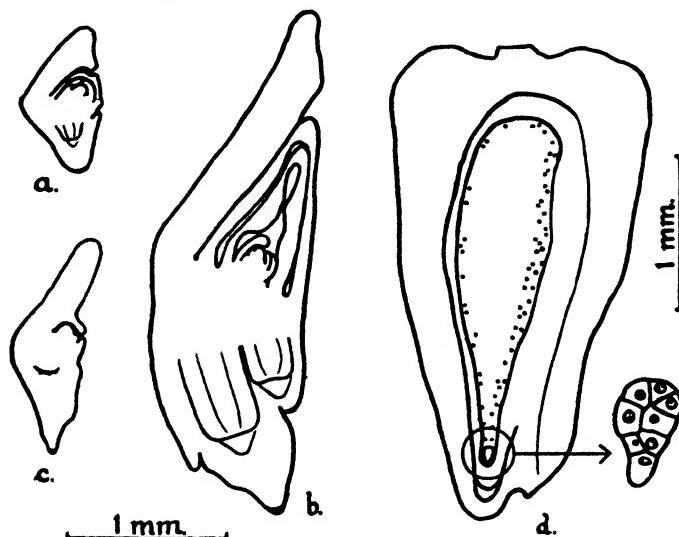
FIG. 4. (*a*) Mature embryo from grain of ear removed five days after anthesis. (*b*) Mature embryo from normally ripened grain. (*c*) Normal embryo nineteen days after anthesis. (*d*) Embryo-sac and embryo five days after fertilisation.

The results shown in Figs. 1–3 emphasize two points: first, that both embryo size and grain size depend directly on the time of harvest, and second, that in ears harvested when ripe, as well as in the experimental material here used, there is a normal distribution of size in the grain. 'Dwarf' and 'normal' are therefore relative terms and in the following account a definite comparison is made between embryos from grain harvested five days after anthesis and embryos from grain harvested thirty days after anthesis.

The observation by Gregory and Purvis (1938) already referred to, that ears of rye removed as early as five days after fertilization and dried down produced viable grain, was confirmed, but in no case was dwarf grain obtained from ears removed at an earlier stage than five days after anthesis.

Returning to the comparison of normal and dwarf embryos, in Fig. 4 are shown camera lucida drawings of median longitudinal sections of (*a*) a 'five-day' and (*b*) a normal (30-day) embryo.

The linear dimensions are approximately in the ratio of 1 : 3·5. Models were made of these embryos by superimposing profiles of serial sections cut from paraffin wax plates of appropriate thickness. The volumes of the models so obtained were in the ratio of 1 : 12.

Fig. 4 (*d*) represents a median longitudinal section of the carpel five days after fertilization. The embryo is quite undifferentiated and contains about

sixteen cells; endosperm development is still in the free nuclear stage and the antipodal cells have just completed their degeneration (Nutman, 1939). The figure serves to emphasize that in the ears harvested prematurely most of the development of the embryo and endosperm takes place during the process of drying down.

In the same figure is also shown (*c*) a median longitudinal section of a stage of the development of a normal embryo in which it is equal in size to the mature dwarf (*a*). It will be evident that dwarfing has not resulted from a mere *arrest* of normal development since the development of the dwarf embryo is complete; nor is it due to a reduction in cell size. Cell size was estimated on stained sections of embryos by counting the number of nuclei seen under the microscope in a field 50 μ square on sections 10 μ thick. The mean cell size for each part was calculated in cu. mm. and it was found that cell size is the same in homologous regions in both types.

The results are shown in Table I below:

TABLE I

Mean cell size (cu. mm. $\times 10^{-6}$.)

Region of embryo.	Normal embryo.	Mature embryo from ears removed 5 days after anthesis.
Scutellum . . .	4·17	3·85
Stem Apex . . .	1·04	1·09
Root	1·74	2·03
Coleorhiza . . .	3·85	4·17

The differences in size of the embryos is therefore due to difference in cell number only.

In general the morphology of the two types of embryos is similar, but the number of embryonic organs is less in the dwarf. The normal embryonic plumule consists of coleoptile, stem apex, three or four primordia of foliage leaves, and possibly two tiller primordia; whereas the dwarf embryo possesses only two foliage-leaf primordia without tiller primordia. Also the mature normal embryo has a complement of four secondary roots in addition to the primary radicle, whereas the dwarf embryo has only the primary radicle.

It will be noted from Fig. 4 that in the dwarf embryo all these organs, with the single exception of the stem apex itself, are reduced in size, so that the dwarfing of the embryo has not resulted from the suppression of one part at the expense of another; and moreover, the relative sizes of these organs are the same in both the normal and dwarf embryos. By the measurement of a number of examples it was established that it is only in the stem apex that the dimensions remain the same irrespective of the size of the embryo. The absence of dwarfing in the stem apex may be related to the fact that cell size is itself independent of embryo size. It is well known that differentiation at the stem apex is controlled by a size relation; lateral appendages are formed with a

'bulk ratio' characteristic of the species. It is likely that in the dwarf embryo the rate of cell multiplication is reduced so that this value is less frequently attained, or that in the desiccated ears dormancy ensues at an earlier stage. Thus the development of the lateral organs of the plumule is suppressed though the size of the stem apex itself does not alter. Summarizing, therefore, it can be stated: (1) that cell size and the dimensions of the stem apex are independent of embryo size, and (2) that the number of root apices present and the size of the embryonic organs (excepting the vegetative point itself) are all reduced in proportion to the size of the embryo.

The rest of the caryopsis harvested at a premature stage also differs from that of a normal harvest date. The fruit as a whole is shrivelled, and the aleurone layer is prominently wrinkled and folded so that it is proportionately much greater in extent. The endosperm cells are not packed full of starch as in the normal grain, and its accumulation is more complete at the periphery of the endosperm than at the centre. In the normal grain starch formation begins and is first completed in the central region; the reverse distribution in the dwarf grain may indicate that the premature drying disturbs the translocation processes. The anomalous presence of abundant starch in the shrivelled testa also supports this view.

The date of earliest successful harvest related to features in the normal development of the grain.

Harlan and Pope (1922) found that removal of the ear of barley earlier than five days from anthesis always resulted in the abortion of the ovary. It has been emphasized above that with rye also the removal of the ear earlier than five days after fertilization leads to the death of the ovary. It appears significant that such a 'threshed value' should be obtained in each case. Harlan and Pope's research was undertaken in Idaho where the air is much drier and day temperature much higher than in this country. The conditions of drying of the ears of barley described by Harlan and Pope and of the rye in the present investigation are so different that the time of earliest successful harvest, the same in both cases, would appear to be determined by internal factors rather than by external conditions. It is in this connexion that an examination of the normal development of the embryo is of interest.

Attention has already been drawn to the appearance of embryo and embryo-sac five days after pollination (Fig. 4 *d*). The embryo appears as a small, pear-shaped, quite undifferentiated mass of meristematic tissue, attached to the micropylar end of the embryo-sac. At this stage the embryo consists of about sixteen cells. It increases in size by regular cell division without change in form until nine or ten days after pollination, when the first indication of morphological differentiation appears. The time of earliest successful removal of the ear cannot therefore be correlated with the appearance of any morphological feature of the embryo.

In a previous paper (Nutman, 1939) on the development of the grain of rye the normal growth of the embryo-sac was shown to be discontinuous. There

is first a period of very rapid extension growth lasting to about the sixth day from pollination, followed by a period of three days during which no growth takes place. The final stage of the embryo-sac development begins about the tenth day and is complete about the twentieth day from anthesis. The end of the most rapid phase of the first growth period coincides with the earliest time of removal at which the grain can afterwards be successfully grown; it would appear necessary, therefore, for the first extension phase of embryo-sac growth to be more or less complete before the grain of the detached ear is able to complete its development. Here it should be noted that with rye only a moderate percentage of ears removed five days after fertilization give viable grain, whereas ears which are harvested six days from pollination yield from the spikelets which first reached anthesis a nearly normal percentage. In the latter case the first extension growth phase is much nearer completion at the time of removal. From the third day to the fifth day after fertilization the ovary is increasing in size most rapidly, and almost entirely, by the movement of water into the grain. In material cut four days after anthesis this extension growth cannot proceed normally since the only source of water, the stem and glumes, is presumably insufficient.

Germination and Growth of Normal and Dwarf Grain

Details of experimental arrangements are set out below.

Germination.

The grains were germinated separately in moist sand in the dark in specimen tubes 3 in. × 1 in. Each seed was planted vertically, embryo end downwards so that the top of the seed was level with the surface of the sand. Daily observations were made on the appearance and length of coleoptile and on the emergence of first leaf.

It was found that above a grain weight of 2·5 mg. percentage germination is unaffected by grain size. Of eight grains of 1·5 mg. weight only two, and of seven grains of 2 mg. four, germinated. The percentage germination of the remaining classes was about 80 per cent. No direct correlation was found between grain size and the appearance and length of the coleoptile or the emergence of the first leaf.

In the case of four grains (all less than 3·5 mg. in weight) normal germination failed owing to the inability of the coleoptile to push through the seed coat. The coleoptile in each case elongated within the seed coat until the distal end was reached, in which position it became coiled and distorted by further growth. When the seed coats of three of these grains were slit to release the coleoptile normal germination ensued; the single seed which was left untreated died. The former grains when transferred to water culture developed into normal plants. They were, of course, excluded from the general experiment but were included for the purpose of calculating the percentage germination.

In addition to the above data the length of each grain was measured before germination. Within each class considerable variation in length was found. Individual grain length within each class, however, bore no relation to subsequent germination or to the final weight of the plants after four months' growth.

After germination selected seedlings were transferred to water culture bottles and their fresh weights, tiller numbers, and leaf numbers determined at intervals. The selection was made on a basis of original grain weight to constitute the following classes:

Class number.	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
Number in class	9	12	12	13	12	12	12	12	11
Mean grain weight (mg.)	2·3	3·0	3·5	4·0	7·9	12·0	20·0	27·8	36·3

Nutrient solution.

A culture solution described by Hoagland and Arnon (1938) and of the following composition was at first employed:

Constituents.		Parts per litre of solution.
N. ammonium phosphate, $NH_4H_2PO_4$	. .	1 c.c.
N. potassium nitrate, KNO_3	. . .	6 c.c.
N. calcium nitrate, $Ca(NO_3)_2$	. . .	4 c.c.
N. magnesium sulphate, $MgSO_4.7H_2O$	. .	2 c.c.
5 per cent. ferric chloride, $FeCl_3$	. .	1 c.c.
Boron (as H_3BO_4)	. . . .	0·0005 gm.
Manganese (as $MnCl_2.4H_2O$)	. . .	0·0005 gm.
Zinc (as $ZnSO_4.7H_2O$)	. . .	0·00005 gm.
Copper (as $CuSO_4.5H_2O$)	. . .	0·00002 gm.

The solution was made up as required by diluting a mixture of molar solutions of the chief constituents with tap water. For the supplementary elements a dilute mixture of the necessary salts was made so that the addition of 1 c.c. of the mixture per litre of the final solution gave the correct concentration. The ferric chloride was added to the culture solution the day following the other constituents; the pH was adjusted to 5·8–6·0 with decinormal sulphuric acid. The rather large proportion of calcium in the solution together with the considerable amount of calcium in the tap water was found to be unsatisfactory for rye. After March 31 the ratio of calcium to potassium was reduced to 1 : 24 maintaining the nitrate content at the same level. This change had no effect on the growth rate of the plants ·but improved their appearance by eliminating a tendency to chlorosis.

The solution was aerated every other day, and the solution was changed at intervals depending somewhat on the size of the plants, but frequently enough to maintain the concentration of salts in the solution very near the original level. Any change in volume of the culture solution due to transpiration was made up by frequent additions of tap water. The dates on which

the solutions were changed were: March 20, 31; April 11, 18, 25; May 1, 8, 12, 16, 22, 26, 31; June 5, 9, 19, 30; July 6.

Containers.

For the greater part of the experiment quart glass bottles were used. The plants (one to each bottle) were held in position with cotton-wool in grooved corks which had been previously impregnated with paraffin wax. The bottles were painted black outside to discourage the development of algae, and each bottle was wrapped in white paper to prevent overheating of the contents in the sun. As the plants increased in size the cotton-wool padding was at first reduced, then the holes in the corks enlarged, and later the corks were discarded altogether. Finally when the plants became rather large for the bottles (June 8) they were moved to large eight-litre glazed earthenware pots.

The grain was set to germinate on March 3 and planted in the bottles on March 9. Until April 19 the plants were kept in a dry, slightly heated greenhouse, in which as the season advanced the heat was gradually reduced. From April 19 to April 24 the water cultures were taken outside during the day and brought in at night, but after April 24 they were out of doors permanently.

At the beginning the bottles were packed closely together on a bench in the greenhouse, but as the plants grew the bottles were spaced far enough apart to prevent mutual shading. In order to eliminate any effect due to the relative positions of the plants the latter were randomized every time the plants were weighed or the solution changed.

Weighing.

The fresh weight of each plant was determined weekly from March 9 to April 26, and at about fortnightly intervals subsequently. Before weighing the roots were allowed to drain, and blotted carefully. After weighing, the plants were replaced immediately in the nutrient solution. The roots suffered no noticeable injury from the treatment except towards the very end of the experiment. The method is subject to large errors because of the difficulty of blotting the roots uniformly. The results show, however, that these are not of great significance when the variation in weight within each class is considered. The smallest weights were determined on a torsion balance reading to 1 mg. and the largest on a pair of chemical scales accurate only to 0·5 gm. For the intermediate weights a series of Joly spring balances was found convenient.

It was not possible to take fresh weights after July 4 because at this time many of the plants became affected by a bacterial rot of the roots. Although this often had no marked effect upon the tops whole parts of the root system rotted completely away from the plant and dropped to the bottom of the jar. This would, of course, have invalidated any fresh weights which might have been taken. The rot was equally evident in all classes.

On May 23 and June 20 some of the intermediate classes were excluded from the experiment and the fresh and dry weights of the roots and tops determined separately.

Tiller numbers and leaf numbers (i.e. the number of leaves expanded on the main axis) were noted at the same time as the determination of the fresh weights were made. In the excluded classes the following information was recorded: the total number of leaves developing on the main axis and the length and stage of development of the main meristem.

Growth rates.

The growth curves shown in Fig. 5 A were constructed from the calculated mean fresh weights of each class taken on twelve occasions during the experiment. The results of five only of the original classes are represented. The fresh weights are plotted on a logarithmic scale. Values of fresh weight for all classes at five intervals of approximately four weeks are entered in Table II.

TABLE II

Mean fresh weight (mg.) of plants

Class no.	No. of Replicates.	Mean grain wt.	March 9.	April 3.	April 26–7.	May 23.	July 4.
I.	9	2·3	16	78	1713	16260	124800
II.	12	3·0	24	107	2271	17460	111900
III.	12	3·5	26	117	2424	19230	—
IV.	13	4·0	30	151	2859	22350	140000
V.	12	7·9	57	303	5500	29580	156300
VI.	12	12·0	79	509	7393	35130	—
VII.	12	20·0	102	780	10150	48700	199000
VIII.	12	27·8	136	1022	11213	49110	—
IX.	11	36·3	150	1266	12120	51950	191800

In general terms the curves consist of two parts, a break appearing in all the curves corresponding to the time of removal of the plants into the open. The slope of the curve at any point is a measure of the relative growth rate, and in the case of the normal plants this is approximately constant both before and after removal from the greenhouse. The difference in relative growth rate due to the environmental conditions is not here considered; it is rather to the difference in relative growth rate between classes that attention is directed. These differences are indicated throughout the greater part of the experiment by a general convergence of the curves of growth rate. The plants of class IX, grown from the largest grain, have a high initial relative growth rate which decreases during the second week and then remains at an approximately constant level, apart from the environmental effect. The plants grown from the dwarf grain (class I) possess a low initial relative growth rate which increases gradually until an approximately constant value is reached after the third week, the direct effect of moving the plants from the greenhouse being

neglected. This steady rate is higher than that of the normal grain. The dwarf grain thus gives rise to plants with a higher growth rate than plants from normal grain, and in consequence the difference in size between plants
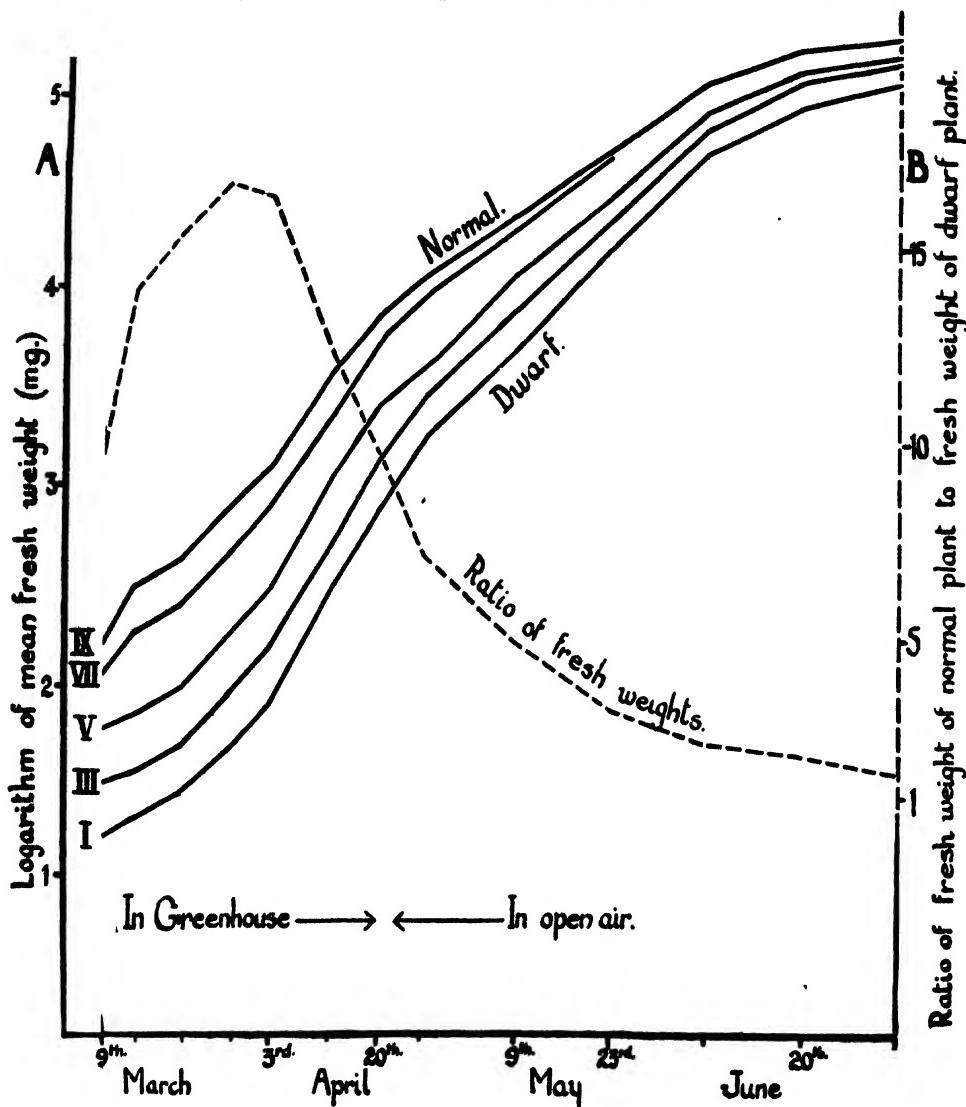
FIG. 5. Growth rates of rye raised from dwarf and from normal grain, expressed on a fresh weight basis. Dotted curve shows the ratio of fresh weights in the extreme classes.

grown from normal and from dwarf grain becomes less and less, until by July 4 it is hardly significant.

The convergence of the growth curves is also shown in Fig. 5 B, in which the ratios of the fresh weights of plants of class IX to those of class I are repre-

sented. At germination the ratio has a value of 9·6, and increases to a maximum of 16·9 at three weeks from germination, thereafter falling at first rapidly and then more slowly to approximately unity. By July 4, when the fresh weights were last determined, the ratio was 1·6. It should also be noted that the plants continued to grow for a further month after July 4 before 'shooting' took place. If fresh weight determinations had been continued a much closer approximation to unity would no doubt have been obtained.

Harlan and Pope (1922) noted that dwarf grain gave rise to apparently normal plants, and give figures in which plants grown from normal and dwarf grain appear to have the same size. Here, however, the plants were grown in pots of soil; several plants were grown in each pot and the result may have been due to the potbound conditions limiting the size of all the plants. The present experiment gives a similar result and shows that the plants from the dwarf grain do in fact grow more rapidly and eventually reach the same size as those from normal grain.

TABLE III

Mean Tiller Number per Plant

Class no.	Mean grain wt. (mg.)	April 12.	April 26–7.	May 23.	June 20.
I.	2·3	0·2	3·4	22·6	79
II.	3·0	0·5	4·7	24·3	83
III.	3·5	0·8	4·3	24·5	—
IV.	4·0	1·2	5·2	29·0	117
V.	7·9	2·1	8·0	34·8	117
VI.	12·0	2·8	9·7	42·3	—
VII.	20·0	4·6	12·7	53·1	150
VIII.	27·8	5·3	13·1	51·4	—
IX.	36·3	5·6	13·3	57·5	113

The rates of tiller and leaf formation.

In Fig. 6 the tiller numbers are shown graphically, and it will be noted that the curves of tiller production in class I (2·3 mg.) and class IX (36·3 mg.) are very similar in form to the curves of fresh weight.

Tillering, like increase in fresh weight, is an exponential process, and its rate is eventually greater in the plants raised from dwarf seed. Also the ratio of tiller number in class IX to that in class I decreases until by July 4 it approximates to unity.

The almost linear relationship between leaf number and date of determination shown in Fig. 7 indicates that increase in leaf number on the main stem takes place by regular addition of new leaves. The differences between the mean leaf numbers of the two classes throughout the experiment is also shown graphically in Fig. 7. This difference rises to a maximum three weeks from germination and then goes on decreasing to the end of the experiment. Thus

at first the rate of leaf production is greater in class IX; at three weeks it is the same in each class and later is greater in the class of smallest original grain weight. The final rate of leaf production is, however, only very slightly greater in class I. During the entire period of the experiment only one extra leaf is differentiated on the main axis of plants of class I as compared with the
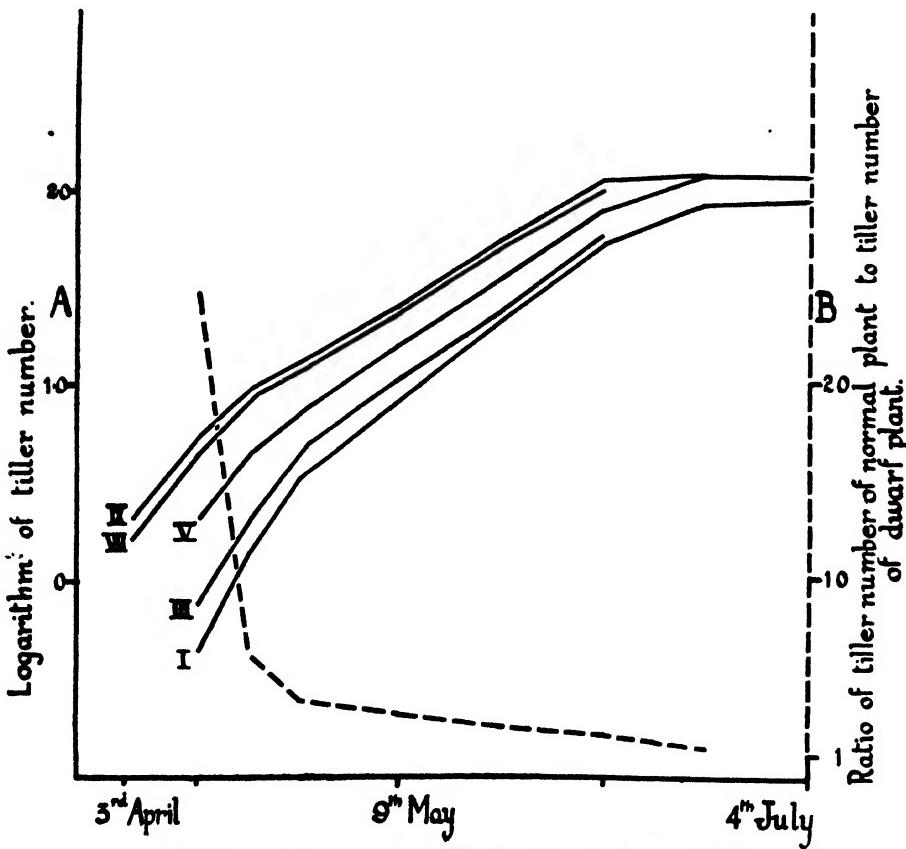
FIG. 6. Rate of tiller formation in rye raised from normal and dwarf grain.

number formed on the plants from normal grain. Since the dwarf embryo has only two leaf initials whereas the normal has three, the increase in leaf production rate in the former case results in both classes having the same mean final leaf number on the main axis.

Data from classes excluded on May 23 and June 20.

In order to obviate some of the objections to fresh weight determinations as a measure of growth, the excluded classes were examined for dry weights of tops and roots as well as for total leaf number and condition of meristem.

The results are shown in Table IV and indicate that (1) the ratio of fresh weights of tops to roots, (2) the ratio of dry weights of tops to roots, and (3) the ratio of total fresh weight to total dry weight are approximately constant, variation being independent of class number and only related to the date of sampling. In this particular experiment, therefore, the fresh weight determina-
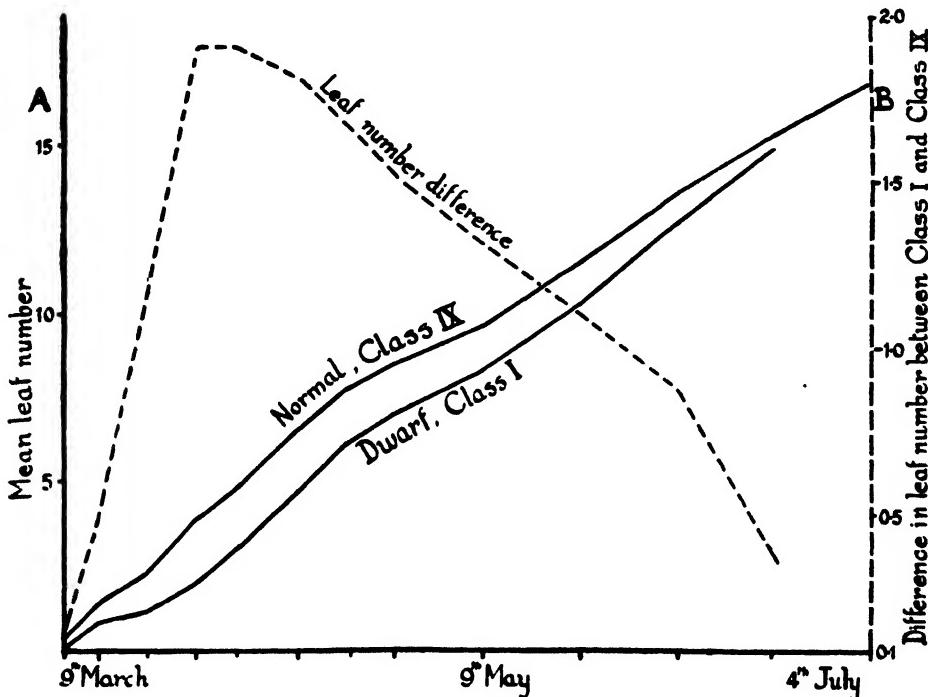
FIG. 7. Rates of leaf formation in rye raised from normal and dwarf grain.

tions give an adequate and not misleading picture of the growth rates of the various classes.

The information obtained from the observations of the total leaf numbers and the condition of the meristem may be briefly summarized. In all classes a final leaf number of between 24 and 26 leaves is attained. By May 23 the earliest stage of the differentiation of the ear was evident, i.e. the formation of double ridges on the meristem (Purvis, 1934). This coincides with the marked decrease in the rate of tiller production in all classes, due allowance being made for the time which elapses between the initiation of a tiller primordium and its appearance.

The progress of development was marked at a later stage by the elongation of the internodes ('shooting') prior to the exsertion of the ears. It may be pointed out that the shooting of these unvernalised winter rye plants was to be expected from the early date of sowing, and the consequent preliminary

TABLE IV

Date of sample.	Class number and original seed weight (mg.)		Number in sample.	Mean fresh weight (gm.)				Mean dry weight (gm.)				Ratio of total fresh weight to total dry weight.
				Roots.	Tops.	Tops/roots.	Total.	Roots.	Tops.	Tops/roots.	Total.	
May 23	III.	3·5	12	6·58	12·67	1·93	19·2	0·47	1·74	3·69	2·21	8·71
May 23	VI.	12·0	12	12·0	23·2	1·94	35·1	0·88	3·33	3·77	4·21	8·35
May 23	VIII.	27·8	12	16·3	32·8	2·01	49·0	1·23	4·82	3·92	6·05	8·10
June 20	I.	2·3	4	25·0	62·4	2·50	89·2	2·31	9·29	4·03	11·85	7·53
June 20	IV.	4·0	7	42·4	88·2	2·08	130·6	3·12	12·9	4·13	16·0	8·15

Date of sample.	Class number and original seed weight (mg.)		Number in sample.	Mean number of leaves fully expanded.	Mean number of leaves fully and partially expanded.	*Condition of meristem, i.e. number of lateral primordia developed.		Mean total number of leaves on main axis.	Meristem length (mm.)
						Simple ridges.	Double ridges.		
May 23	III.	3·5	12	10·5	13·6	9·3†	3·4†	24·3†	0·86
May 23	VI.	12·0	12	10·7	14·7	9·3†	3·6†	24·3†	1·03
May 23	VIII.	27·8	12	11·5	14·5	9·4†	7·4†	24·2†	1·20
June 20	I.	2·3	4	15·0	18·8	6·5	18·8	25·3	1·28
June 20	IV.	4·0	7	14·9	19·0	5·7	23·8	24·7	1·56

* Simple ridges denote lateral primordia which develop into leaves. Double ridges denote primordia which develop into flowers (Purvis 1934).

† The values marked thus were derived from random selections of plants, the numbers sampled being class III 3, VI 3, VIII 5.

exposure to short days (Purvis and Gregory, 1937). On July 12 shooting began in one plant from class I and one plant from class IX. The third plant to shoot, on July 23, was from class VII. By July 26 nearly all the remaining plants were beginning to shoot. The time of shooting differed between the plants by as much as a fortnight but this difference bore no relation to the original grain size; thus harvesting grain at an immature stage has no effect on the date of ear emergence.

DISCUSSION

The data presented show that beyond doubt the rate of growth of the plants derived from the grain of prematurely harvested ears is inversely related to the weight of the mature embryo, the smaller embryos having the higher growth rate. In all cases as seen in Fig. 5 a period of exponential growth covers the phase of vegetative development until flower differentiation begins. Since the plants used are of a winter variety and unvernalised the beginning of the reproduction phase is delayed by the high leaf number (approximately 25) so that the exponential phase extends over nearly four months.

The higher growth rate of the small embryos may be due to one or more of the following causes: (1) a higher assimilation rate, (2) relatively greater development of leaf tissue than of non-assimilating tissue, (3) a relative greater uptake of nutrients by the smaller seedlings, (4) a greater developmental rate. There are no data forthcoming for testing the first three possibilities; only with regard to the last are relevant data available.

With respect both to rate of leaf production and rate of tillering it has been shown that the progeny of small seed show a decided advantage over those of normal size. On these grounds alone the relative growth rate of plants from small embryos must be higher. The plants from small embryos maintain throughout a higher meristematic activity.

With regard to the growth during the first three weeks, when leaf production rate is apparently less in the dwarf class, the following should be noted. First, there are three well-developed embryonic leaves and one or two tiller primordia in the grain from class IX, whereas in the dwarf grain the leaf primordia, two in number, are much smaller and the tiller primordia absent. Second, a point of importance bearing on the preliminary phase is the convention which was adopted in counting the leaves. Each leaf was counted only when the greater part of the leaf blade emerged from the enclosing sheaths of the earlier leaves. A visible unfolded leaf projecting less than an inch from the sheath of the proceeding leaf was counted as 'half a leaf'. The extremely small first and second leaves of the dwarf embryo thus need to make relatively much more growth than those of the normal embryo to reach the stage at which they are counted; thus the retarded rate of leaf production in the dwarf series may be more apparent than real, and may be attributed to the difference in size of the pre-formed leaves in the two cases. Also it may be emphasized that this preliminary phase of growth covers only three weeks; i.e. until the

three embryonic leaves of the normal grain have completed their development. Since the actual stem apex is of equal size in normal and dwarf embryos it is probable that in both cases the later leaves begin their development as lateral primordia of equal size, so that the increased rate of production of leaves in the dwarf class later becomes evident.

An explanation of the greater increase in the fresh weights of class I relative to that of class IX to March 28 also follows from a comparison of the original grain. In the normal grain the first leaves attain a greater final size and at an earlier date, not only because the reserves of the grain are greater, but also because the primordia are initially much larger than those of the dwarf grain, where scanty reserves further restrict growth of the early leaves. Again it is possible that in the dwarf embryo a necessary minimal leaf area has to be produced before active growth of the seedling can proceed and the higher inherent leaf-production rate become manifest.

The preliminary phase as noted above ends abruptly at three weeks from germination, at which time the three seed leaves of the normal grain have completed their development; it is at about this time also that the endosperm is exhausted.

The whole progress of the growth of the dwarf and normal grain may thus be accounted for by the observed fact of greater meristematic activity of the former. As to the cause of the higher meristematic activity of the small embryos little can at present be said. The absence or marked reduction in mass of the endosperm of dwarf grain precludes any simple hypothesis of hormone supply from this tissue, since the dwarf embryos would presumably be less provided than are the normal.

The results of the above experiment are in general agreement with a number of similar studies on the effect of seed size on yield, growth rate, &c., apart from the work already cited on the effect in itself of premature harvest. These other studies are of two kinds: those dealing with the relation of seed size to plant growth from an agronomic and physiological point of view, and others concerned also with the genetical implications of this relationship, and particularly with its bearing on the interpretation of hybrid vigour on a basis of embryo size.

References of the first kind are scattered widely through the literature; summaries may be found in papers by Kidd and West (1919) and Kotowski (1926). The latter conducted experiments which showed that for peas, beans, and cabbages, seed size influenced seedling size only in the early stages of growth, the effect subsequently disappearing. A similar result had been obtained previously by Brenchley (1923), with peas and wheat grown in water culture. In Brenchley's experiment seed size had a proportionate influence on the yield only if the period of growth was of comparatively short duration or the conditions of the experiment severely limited, as for instance with a nutrient solution insufficient for normal growth. Although with annual peas and spring cereals the heavier seed resulted in a larger yield, the

advantage of heavier seed became less if the plants were allowed to grow to maturity. It was suggested that with perennial crops there would be no advantage in sowing heavier seed; a conclusion borne out by the present investigations. Only towards the end of the life history of the winter cereal here considered are the differences due to initial seed weight more or less completely obliterated.

The effect of limiting conditions has also been shown in Phaseolus, by Rudolfs (1923), in experiments on the growth of plants from seed of varying size, in the dark under constant conditions of temperature and humidity. For the early stages of growth studied it was shown that the larger seed gave rise to larger plants which exhibited a higher growth rate than those from smaller seed.

In field experiments Kiesselbach (1924) obtained slightly reduced yield of winter and spring wheat and of oats from seedlings with smaller grain, but not in proportion to the original differences in seed size. Here also limiting conditions of growth, competition from weeds, &c., may account for the small final difference shown.

Percival (1921) reports the results of five years' work with three varieties of wheat using grain of 50 and 25 mg. weight, in which the increases in yield of the larger over the smaller ranged from 37·5 to 50 per cent. The author states, however, that 'where the land has been in specially high condition small seed grain has given remunerative results almost equal to those in which larger grain has been grown.'

In conclusion mention must be made of the bearing of the present work on the theory of hybrid vigour, put forward by Ashby (1930, 1937), in which heterosis is attributed to a larger embryo size in the hybrid; the initial advantage of a larger embryo being maintained by the inheritance as a simple Mendelian character of a constant relative growth rate from one parent.

This interpretation has been called in question by a number of investigators. Passmore (1934), working with Cucurbita, Sprague (1936) with maize, and Luckwill (1939) with tomato, were able to obtain reciprocal hybrids, of identical genetical constitution but of different initial embryo weight, which did not differ in final size. In these cases the advantage of a larger embryo size disappeared during the growth of the plants.

Sprague also obtained slightly smaller embryos by premature harvest; during subsequent growth the initial advantage held by the normally harvested material was soon lost. Further evidence has been supplied by Fabergé (1936) in connexion with a study of the growth of tetraploid and diploid tomatoes. It was shown that an initial advantage of 30 per cent. in the embryo weight of the tetraploid was lost during germination.

In most of the above investigations it is not clear how the initial differences are made up; whether differences in growth rate or in the period of growth are the important factors which make good, during growth, the initial discrepancy. Hatcher (1940) using tomatoes has shown that small seed gives

rise to plants with higher relative growth rate, thus confirming the results of the present work.

These examples could be multiplied, but are sufficient to establish the inverse relationship between seed size and relative growth rate. The results of the present investigation emphasize this relationship by presenting an extreme case, in which the divergence in seed size is very much greater than in any previous investigation.

SUMMARY AND CONCLUSIONS

1. The formation of dwarf grain of rye in ears harvested at an immature stage is described: and the observations made by Harlan and Pope with barley, and by Gregory and Purvis with rye, are confirmed, namely that ears removed from the plant as early as five days after fertilization produce viable grain.

The morphology and anatomy of dwarf and normal grain and embryos are compared, the following being the more important results:

2. The smallest viable grain is $\frac{1}{16}$ the weight of the normal grain.

3. The ratio of embryo weight to grain weight is the same in dwarf as in normal grain.

4. The anatomy and morphology of dwarf and normal embryos are similar, but the dwarf embryo differs from the normal in that it has only two leaf primordia instead of three; also the dwarf embryo has merely a single primary radicle whereas the normal has a complement of five seminal roots.

5. All the embryonic organs of the dwarf embryo, with the exception of the stem apex itself, are reduced in size proportionately; thus the dwarfing does not result from the suppression of one part at the expense of another.

6. Cell size is the same in homologous parts of dwarf and in normal embryos, so that difference in size is due to difference in cell number.

7. The stem apices of normal and dwarf plants are of the same size.

8. The earliest time at which ears may be removed from the plant to give viable grain is five days after fertilization, and this coincides with the end of the phase of first extension growth of the embryo-sac development.

The germination and growth of normal and dwarf grain was examined under the controlled conditions of a water culture experiment.

9. It was found that, over the greater part of the duration of the experiment, the plants grown from dwarf grain exceed those grown from normal grain in (i) relative growth rate (on a fresh weight basis), (ii) rate of tiller production, and (iii) rate of leaf formation.

10. As a result all plants whatever their original grain weight reached approximately the same size at the termination of their period of growth.

11. The three main results noted above are attributed to a single effect, namely to the rate of development of the meristem of plants grown from dwarf grains being higher.

12. The relationship between embryo size and subsequent growth rate here described is contrary to that postulated by Ashby's theory of hybrid vigour.

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Spontaneous Hybrids between Sonchus asper and S. oleraceus

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With six Figures in the Text

SONCHUS OLERACEUS L. and *S. asper* Hill are closely related in morphology. *S. asper* differs from *S. oleraceus* in its more spiny, thicker, and often darker green leaves. It is usually a stouter and more compact plant than *S. oleraceus*. But the most important differences are in the shape of the auricles and the structure of the fruit. In asper the auricles are nearly circular, with a spiny margin and closely appressed to the stem (Fig. 1). In oleraceus the auricles are spreading, arrow-shaped and often deeply toothed (Fig. 3). The fruit in asper is without the transverse ribs of oleraceus. In most of the recent floras the species are separated, although in some of the earlier ones (e.g. Bentham and Hooker) asper is considered as being only a 'marked variety'. Both species are annual and very common weeds of arable land in all temperate regions of the world.

Hybrids between the two have been reported previously by Hegi and in the London Catalogue. They are said to be rare. Both species are self-fertile.

S. oleraceus occurs in two forms distinguished by chromosome number. A diploid has been reported by Marchal (1920) with $2n = 16$ chromosomes, and a tetraploid with $2n = 32$ by Ishikawa (1911) and by Cooper and Mahony (1935). The Merton plants were of the tetraploid type (see Fig. 6), which seems therefore to be the more widely distributed.

The chromosome number of *S. asper* has not previously been determined. Two families, consisting of 12 and 23 plants, were raised at Merton from open pollination of two typical plants of *S. asper*. The seedlings in the larger family were all likewise typical *S. asper* and fertile. The somatic chromosome number was 18 (Fig. 4). Evidently they were diploid, with a basic number of 9. In the smaller family there were three typical *S. asper* plants, which were also fertile. The chromosome number was again 18. The remainder showed some resemblance to *S. oleraceus*. Their habit was looser and more branched, and the leaves were hardly more spiny than the typical *S. oleraceus*. The auricles of the later leaves, however, were more like those of *S. asper*, except that one tooth was much bigger than the others and projected backwards (see Fig. 2).

These intermediate plants were quite sterile both as male and female. The chromosome number was 25 (see Fig. 5). They were undoubtedly hybrids between the two species, with 9 *S. asper* and 16 *S. oleraceus* chromosomes.

No further examples of the hybrids have been found, although several
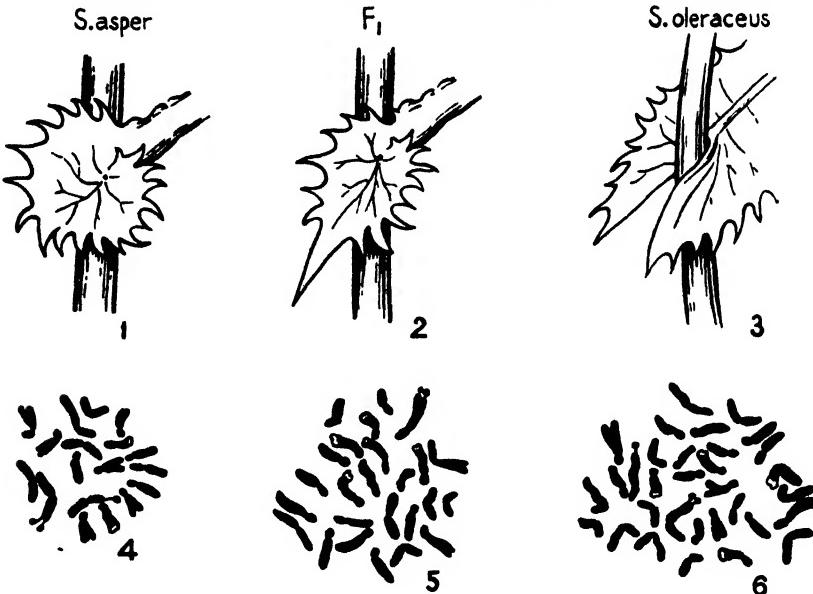
FIGS. 1–6. Figs. 1–3. Leaf auricles of *Sonchus asper*, *S. asper* × *S. oleraceus*, and *S. oleraceus*. Figs. 4–6. Somatic chromosome plates of *S. asper*, *S. asper* × *S. oleraceus*, and *S. oleraceus*. × 3000.

populations of the parent species growing together have been examined for two successive years. Possibly their rarity is due to the ease with which selfing occurs. Attempts to cross the species artificially were unsuccessful.

Comparison of Sonchus asper, S. oleraceus, *and their Hybrid*

	Sonchus asper	Hybrid	S. oleraceus
Habit . . .	Compact	Loose	Loose
Leaf margin . .	Very spinous	Slightly spinous	Slightly spinous
Auricles . .	Circular appressed	Intermediate	Sagittate spreading
Achenes . .	No transverse ribs	(Sterile)	Transverse ribs
Chromosomes .	$2x = 18$	$3x = 25$	$4x = 32$
	$x = 9$		$x = 8$

SUMMARY

Spontaneous hybrids between *Sonchus asper* and *S. oleraceus* are described. The two species differ in their basic number and polyploidy. The hybrids are sterile.

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Studies in Tropical Fruits

XII. The Respiration of Bananas during Storage at 53° F. and Ripening at Controlled Temperatures

BY

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With twenty-six Figures in the Text

I. INTRODUCTION

IN an earlier contribution (Wardlaw and Leonard, 1940) the results of experiments on the respiration of bananas at tropical temperatures were given in some detail on the contention that the results were applicable, *mutatis mutandis*, to respiration at lower temperatures and therefore to the refrigerated transport of bananas which results in an extension of the pre-climacteric phase of partially-developed fruit. The present paper deals with corresponding data obtained from Gros Michel bananas of two commercial

C C

grades of maturity[1] during a period of storage at 53° F., the temperature used in overseas transport, and subsequent ripening at 68° F. or 65° F., these temperatures again following commercial practice. Continuous storage at 53° F. has also been used to examine the phenomenon of 'chilling' as produced by prolonged exposure to this temperature. The methods and materials are those described in earlier papers (Wardlaw and Leonard, 1939; Wardlaw, Leonard, and Barnell, 1939). The rates of respiration measured are those of individual 'fingers',[1] i.e. of single fruits.

II. Respiration Rate of '¾-Full' Fruit

(a) *First at 53° F. and ripened at 68° F.*

Fig. 1 gives data for the respiration rate of a '¾-full' fruit placed at 53° F. immediately on arrival at the Research Station (about 6 hours after harvesting) and maintained at that temperature for 15 days, and subsequently at 69° F. until final senescence. The atmosphere in the respiration chamber was at 100 per cent. R.H. throughout. During storage at 53° F. the respiration maintained a steady rate after a slight initial fall. On transference to 69° F. there was a well-marked transition effect, i.e. a rapid temporary increase in CO_2 output followed by a slow decline, and thereafter a steady rate was maintained for a further 10 days, at which stage the fruit was still green and firm. With the onset of the climacteric the respiration rate rose to a peak value, descended to a new high level, again rose slightly, and finally fell off steadily on the completion of the senescent phase. Annotations indicate the various ripening changes observed. Relevant data are cited in Table I.

Table I

	Fruit No. 7[2] (that of Fig. 1).	Fruit No. 3.[2]	
Initial weight of fruit	132·00	135·08 gm.	
Respiration rate at 53° F.	13	15	mg./kg./hr.
Respiration rate at 60° F.: preclimacteric phase	21	24	,, ,,
Respiration rate at climacteric peak . .	150	149	,, ,,
Respiration rate during post-climacteric peak .	100–120	110–135	,, ,,
Respiration rate during final senescence .	95	80	,, ,,
Time to reach climacteric peak from initiation of ripening	54	62	hours
Time to reach climacteric peak from commencement of experiment . . .	31	27½	days
Duration of experiment	46	43	,,
Final weight of fruit	119·25	121·25 gm.	
Loss in weight (% of initial weight) . .	9·66	10·24	
Final pulp/skin weight ratio . . .	2·24	2·50	

Fig. 1 also shows the transpiration (i.e. total loss in weight) rate curve for the same fruit; this is discussed in a later section. Values for the temperature of

[1] For explanation of terms see Wardlaw, Leonard, and Barnell (1939).
[2] The fruits are numbered from left to right in the hand, viewed from the proximal end and omitting the atypical outer finger.

the air in the respiration chamber and for the carbon dioxide concentration, taken at intervals, are also given.

(b) *At 53° F. throughout.*

Fig. 2 gives data similar to those of Fig. 1 for a comparable fruit maintained throughout at 53° F. (approx.) and 100 per cent. R.H. The onset of the climacteric after 34 days is denoted by a rise in respiration rate from 15 to 37 mg./kg./hr. in the course of 7 days. Thereafter the respiration rate shows a slow decline to a value of 24, then rising slowly to 35 mg./kg./hr. during final senescence; annotations indicate the various ripening changes observed. Additional data are cited in Table II.

TABLE II

	Fruit No. 5[1] (that of Fig. 2).	Fruit No. 1.[1]
Initial weight of fruit	136·10	126·76 gm.
Time to reach climacteric peak	33½	34 days
Time from initiation of ripening . . .	5	7 ,,
Time to reach 'sprung' condition . . .	48	50 ,,
Time to reach 'eating-ripe': fruit showing full 'chill' yellow	57	55 ,,
First appearance of anthracnose . . .	59	60 ,,
Final weight of fruit	123·67 (at 106 days)	110·67 gm. (at 105 days)
Loss in weight (% of initial weight) . . .	9·14	12·68
Final pulp/skin weight ratio	2·47	2·30

A special interest attaches to this and similar fruits which were kept under observation because of the data they have yielded on the subject of chilling and ripening during continuous storage at 53° F., the usual commercial storage temperature for the Gros Michel banana. This is discussed in section VII.

Fig. 2 also shows the curve of transpiration rate for the same fruit and of temperature and carbon dioxide concentrations in the respiration chamber. It may be noted that at the two temperatures the climacteric rise began at approximately the same time, i.e. 31 days (Fig. 1) and 29 days (Fig. 2) from the commencement of the experiment. The time to reach the climacteric peak from the onset of ripening was, however, about twice as long at the lower temperature.

III. INTERNAL CONCENTRATIONS OF CO_2 AND O_2, AND CO_2 CONTENT OF '¾-FULL' FRUIT

(a) *Uniformity of material.*

For the observations recorded in this section top row fingers from two hands of one bunch and from one hand of a second bunch (that from which the fruits used in section II above were taken) were detached and supplied with gas-sampling tubes at the distal end for observation of internal gas con-

[1] See footnote 2 on opposite page.
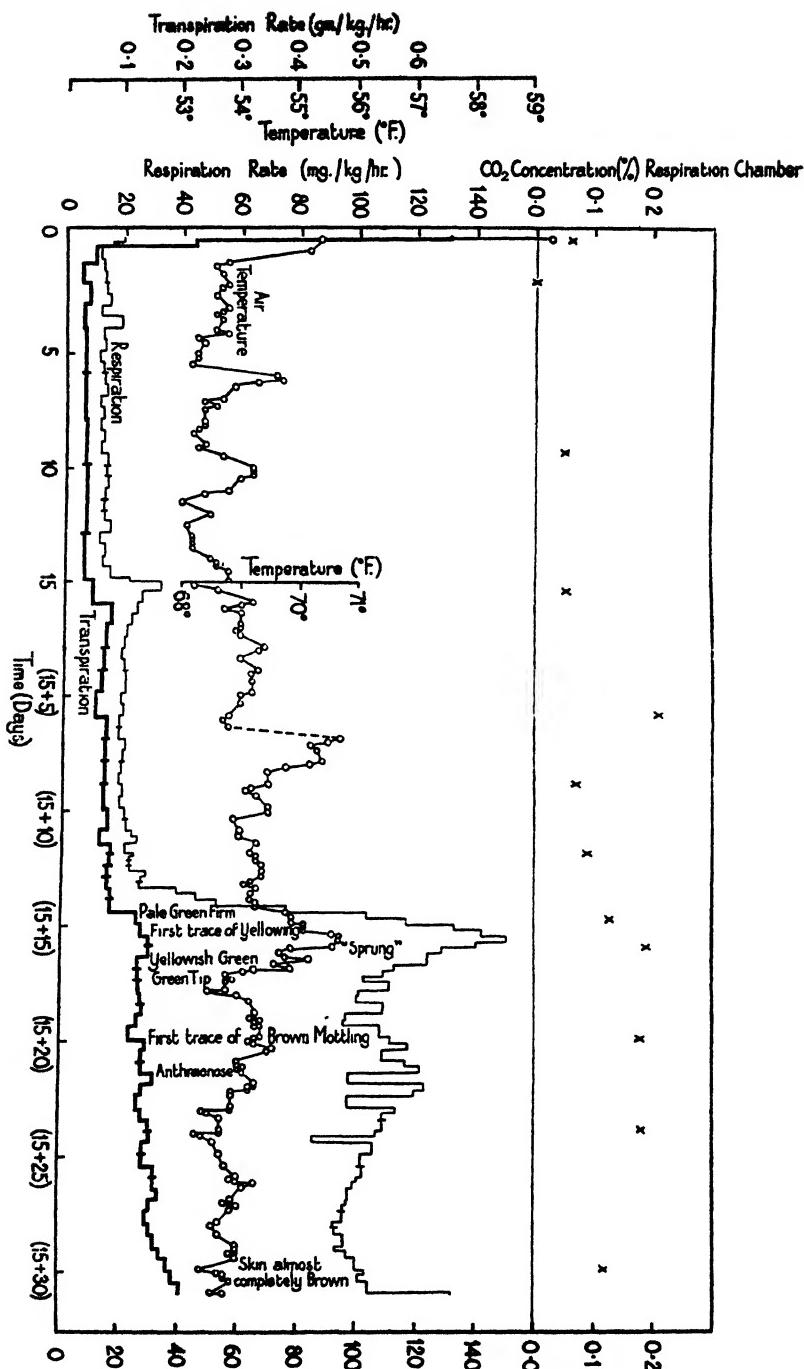
FIG. 1. Respiration and transpiration rates of a '¼-full' banana at 53° F. (approx.) and 100 per cent. R.H. for 15 days followed by ripening at 69° F. (approx.). Temperature of air in the respiration chamber by point observations of mercury-in-glass thermometer. Point observations at intervals of carbon dioxide concentration (per cent.) in respiration chamber air.

centrations (Wardlaw and Leonard, 1939). The fruit was maintained at 85° F. for 24 hours and thereafter placed at 53° F. and 85 per cent. R.H. For records of the latter temperature and humidity see Fig. 23.

At the beginning of the experiment observations were made on a total of 23 fruits. Figs. 3 and 4 give some indication of the range of variation in respect of internal gas concentrations of fruit from the three hands. Fig. 3 shows all the observations obtained during 24 hours at 85° F. and during 14 days at 53° F. The limits of carbon dioxide and oxygen concentrations observed are outlined; in Fig. 4 curves for the mean internal concentrations of CO_2 and O_2 are shown for the fingers from each of the three hands during this period.

(b) *At 53° F. and ripening at 68° F.*

The internal concentrations of CO_2 and O_2 at 85° F. were of the order already established for the grade of fruit (Wardlaw and Leonard, 1940), i.e. CO_2, 2·0 per cent. to 2·8 per cent. and O_2, 12·8 per cent. to 14 per cent. On transferring fruits to 53° F. the internal concentration of CO_2 fell to a mean value of approximately 1·0 per cent. while that of O_2 rose to a mean value of 18 per cent. From such observations it is apparent that when fruits are placed in cold storage marked changes are effected in their internal gas concentrations. The importance, as a first step, of analysing the purely physical aspects of such a change has already been indicated in a preliminary paper on gas solubility phenomena in relation to respiration (Leonard, 1939). Lowering the temperature increases to a different degree the solubility of CO_2 and
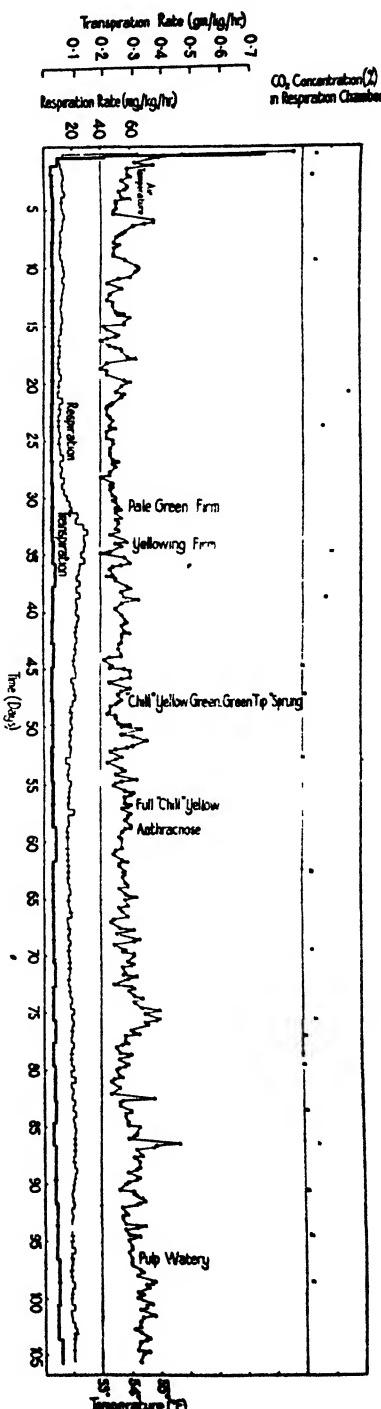
FIG. 2. Respiration and transpiration rates of a '½-full' banana at 53° F. (approx.) and 100 per cent. R.H. continuously. Temperature and carbon dioxide concentrations of respiration chamber air as in Fig. 1.
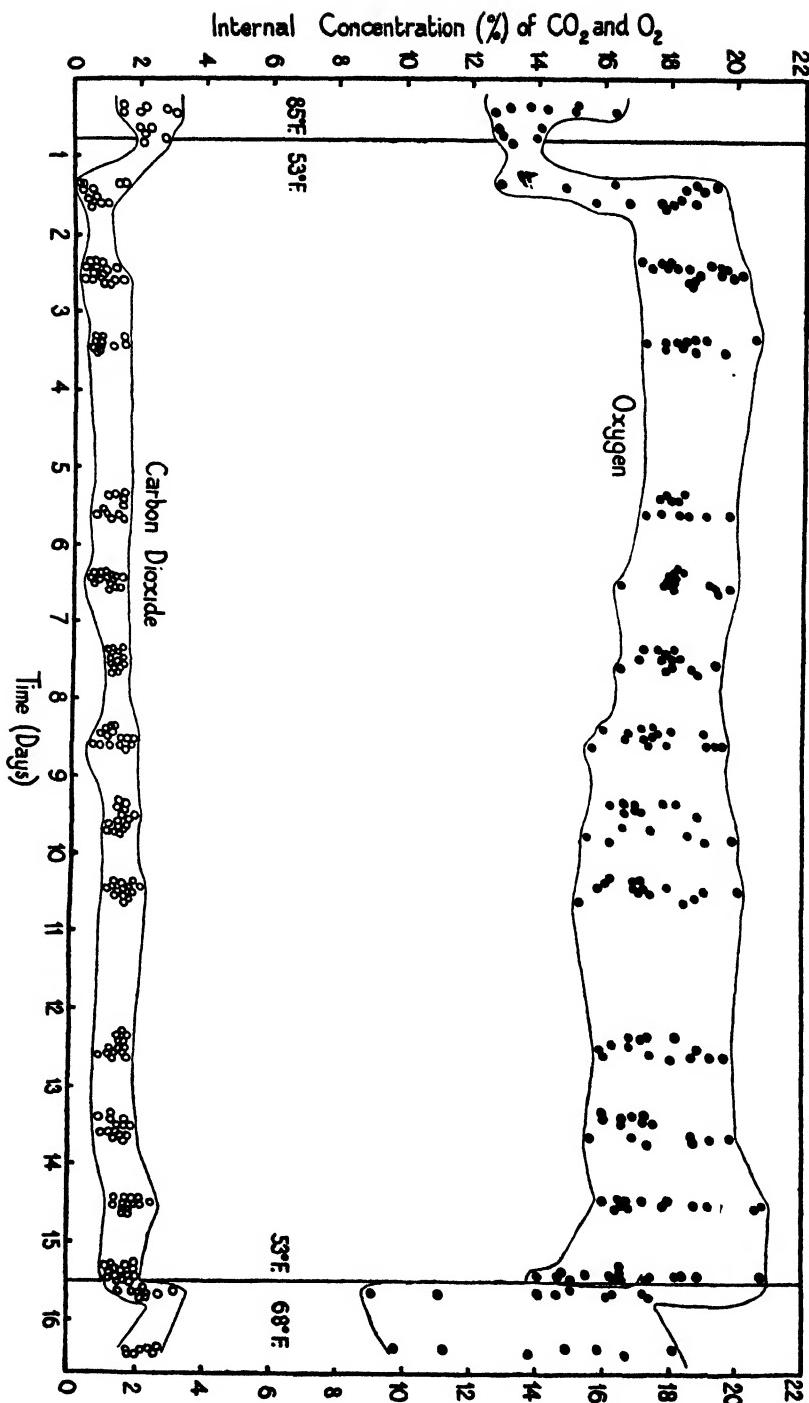
FIG. 3: Percentage internal concentrations of CO₂ and O₂ at 85° F., 53° F., and 68° F. and 85 per cent. R.H. for fingers of top row from 3 hands of '½-full' bunches. The thin lines denote the limits of concentrations observed.
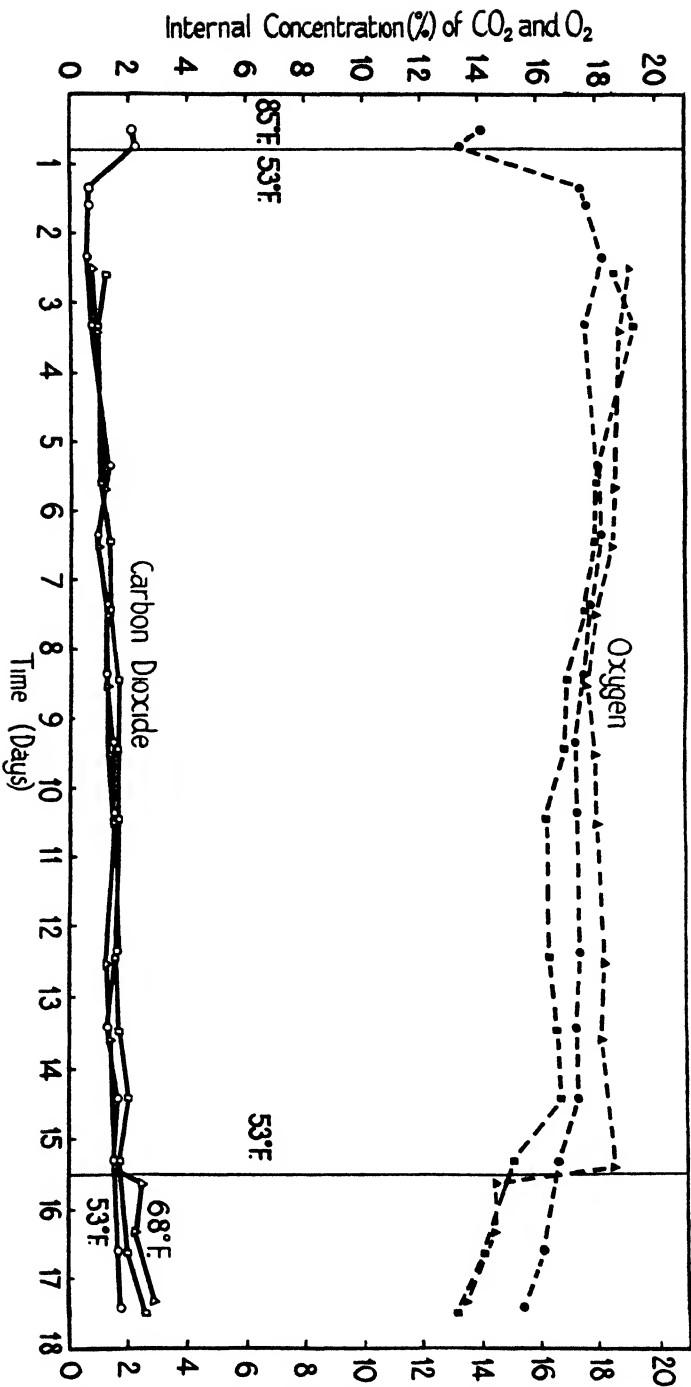
FIG. 4. Mean percentage internal concentrations of CO_2 and O_2 at successive temperatures of 85° F., 53° F., and 68° F. and 85 per cent. R.H. for fingers from three similar hands from '¾-full' bunches of bananas. Only fruits denoted by △s were transferred from 53° F. to 68° F. after 15 days.

O_2, and at the same time the rate of metabolism is reduced involving diminished production of CO_2 and utilization of O_2. As a result a new state of equilibrium is produced involving an increase in the internal concentration of oxygen by approximately 50 per cent. and a decrease of the internal concen-
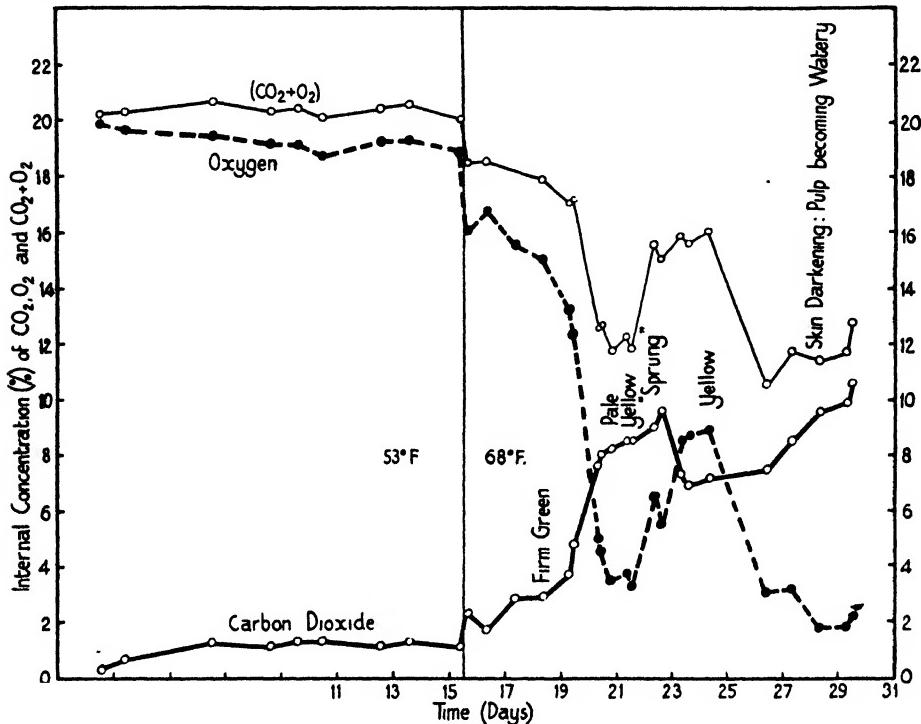
FIG. 5. Percentage internal concentrations of CO_2, O_2, and (CO_2+O_2) for a '¾-full' banana (No. 22) at 53° F. and 85 per cent. R.H. for 14½ days followed by ripening at 68° F. and 85 per cent. R.H.

tration of CO_2 by 100 per cent. These changes in the internal gas concentrations, in particular that of oxygen, will in turn react on the metabolic changes in progress and may account in part for modifications in the metabolic trend during ripening; they may also be associated with the production of the abnormal changes observed where chilling injuries have been sustained. The changes in the internal atmospheres of fruits on being placed in cold storage must also be taken into account in investigations on storage where controlled atmospheres are employed as in gas storage.

Fig. 5 shows the internal concentrations of CO_2, O_2, and CO_2 *plus* O_2 for a single fruit at 53° F. for 14½ days with subsequent ripening at 68° F.[1] As in Figs. 3 and 4, approximately steady levels are shown for the internal concentrations of CO_2 and O_2 at 53° F. On transference to 68° F. a transition

[1] In Fig. 23 temperature and humidity conditions of this fruit and those of Fig. 6 for the first 15 days are recorded, and subsequent data are shown in Fig. 22.

effect is observable in the curves for both CO_2 and O_2, the former rising rapidly and then falling, the latter falling and then rising. This transition effect was followed within two days by the onset of ripening involving a rise in the internal concentration of CO_2 and a fall in the concentration of O_2.
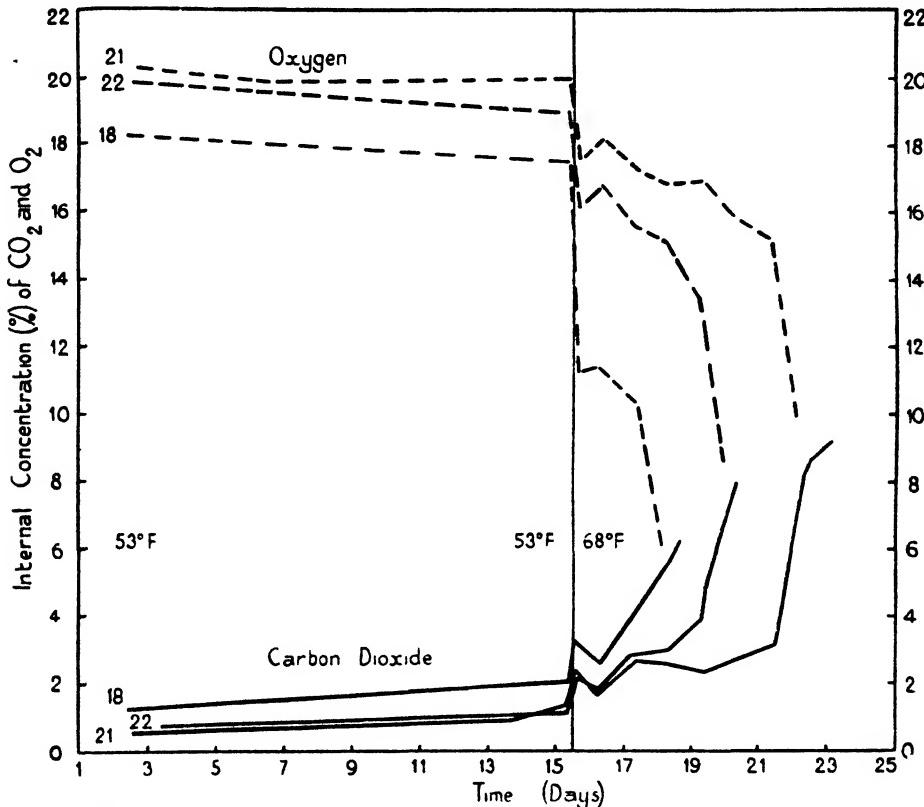
FIG. 6. Percentage internal concentrations of CO_2 and O_2 for three '¾-full' bananas (Nos. 18, 21, and 22) at 53° F. and 85 per cent. R.H. for 15 days with subsequent ripening at 68° F. and 85 per cent. R.H. Transition effects are seen on transference from the lower to the higher temperature: thereafter the rapid rise in CO_2 and fall in O_2 indicate the onset of the climacteric for each fruit. Fruit numbers as in text.

(The considerably later ripening of the fruit of Fig. 1 is discussed later.) The subsequent trend and time relationship of the curves of internal gas concentrations were similar to those obtained at tropical temperatures (Wardlaw and Leonard, 1940), with an extended time basis during ripening at the lower temperature. Annotations show the several ripening changes.

The part which oxygen may play in the initiation of ripening changes has already been briefly considered (Wardlaw and Leonard, 1940). The trends of the curves of internal O_2 concentration obtained during the present studies also indicate its importance. Fig. 6 shows the trends in internal concentration of CO_2 and O_2 for three fruits (Nos. 18, 21, and 22) at 53° F. with subse-

quent ripening at 68° F.[1] It will be noted that one fruit (no. 18) with the lowest
internal O_2 concentration at 53° F. showed the greatest fall in this concentra-
tion on transference to 68° F., and was also the first fruit of this series to show
ripening changes. Fruit 22, the complete record of which is given in Fig. 5,
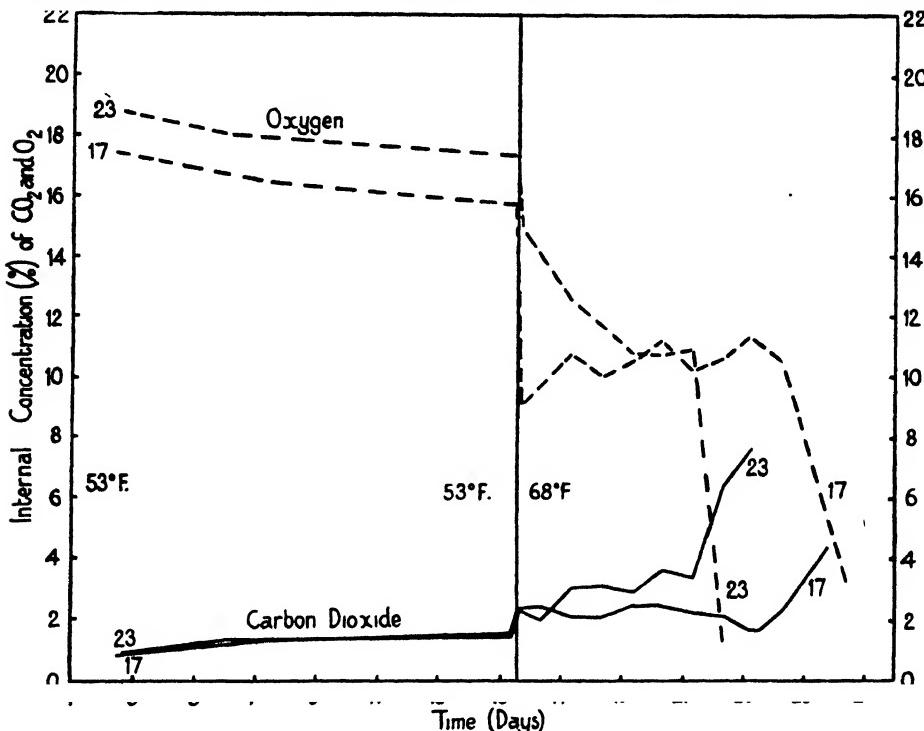
FIG. 7. Percentage internal concentrations of CO_2 and O_2 for two '$\frac{3}{4}$-full' bananas at 53° F.
and 85 per cent. R.H. for 15 days with subsequent ripening at 68° F. and 85 per cent. R.H.
Fruit numbers as in text. Compare with Fig. 6.

occupied an intermediate position between Nos. 18 and 21 in respect of its
internal gas concentrations, showed an intermediate fall in O_2 concentration
on transference to 68° F., and occupied an intermediate position in respect of
the onset of ripening. Such observations might be interpreted as indicating
an active relationship between critical oxygen concentrations and the initia-
tion of the climacteric, but other observations on the internal oxygen concen-
trations of fruits 17 and 23 from the same series (Fig. 7) do not support this
view.

CO₂ content of tissue. Using the method and system described in earlier
papers (Wardlaw and Leonard, 1939 and 1940) the CO_2 content of skin and
pulp was estimated for fruit selected at different stages during the course of
the experiment. Fig. 8 shows comprehensively the data obtained. The
composite curves (A) of internal concentration of CO_2 and O_2 are in general

[1] See footnote, p. 386.

agreement with the curves obtained from a single fruit, Fig. 5. The curve B of Fig. 8 gives the total CO_2 content of a fruit of 140 gm. weight and it will be noted that constant values were obtained at 85°, 53°, and 68° F.[1] until the onset of ripening when the curve rose to a peak value coinciding with the
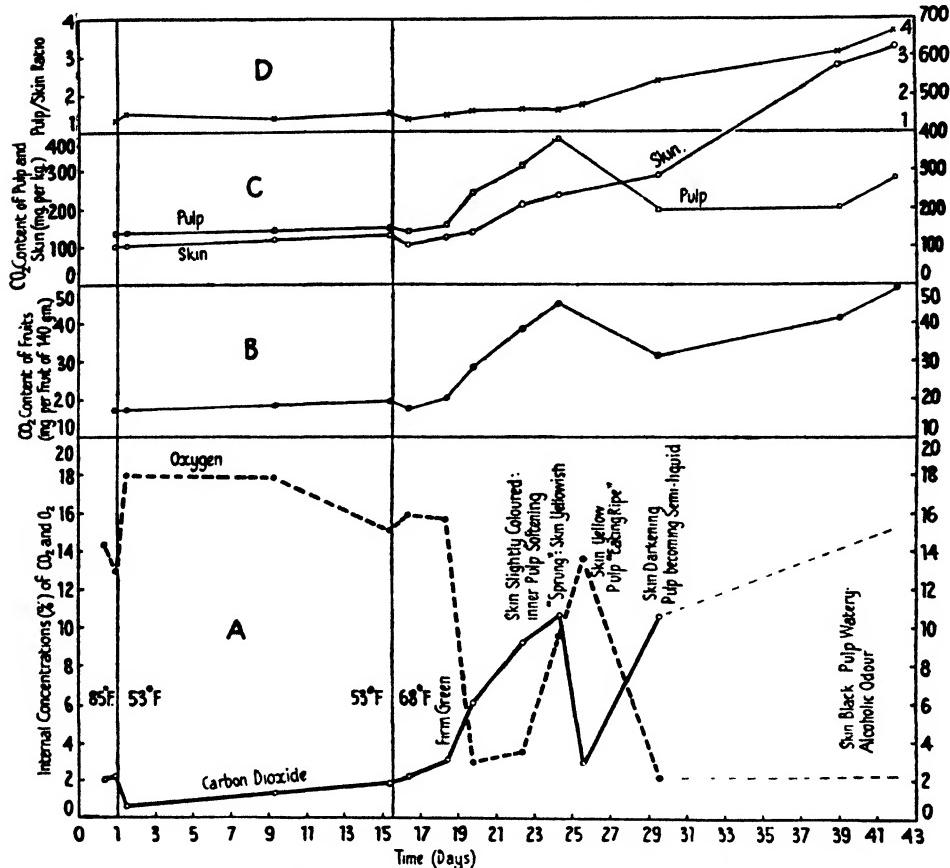
FIG. 8. Composite curves (see text) for '$\frac{3}{4}$-full' bananas at 85° F. for a preliminary period followed by 15 days at 53° F. and 85 per cent. R.H. with subsequent ripening at 68° F. and 85 per cent. R.H. A. Percentage internal concentrations of CO_2 and O_2. B. CO_2 content of fruit. C. CO_2 content of pulp and skin. D. Pulp/Skin weight ratios.

peak value of the curve of internal concentration of CO_2. Thereafter the curve of CO_2 content declined but subsequently rose during final senescence, this trend being similar to that obtained for fruit held at tropical temperatures. Curves C of Fig. 8 show the CO_2 content of pulp and skin as mg./kg. of each tissue. During the preclimacteric and climacteric stages the CO_2 content of the pulp was consistently higher than that of the skin, but during the post-climacteric phase the curves show a cross-over, very high values, on the basis

[1] Temperature and humidity records for the first 15 days are given in Fig. 23, and subsequently in Fig. 22.

of fresh weight, being ultimately recorded for the skins at the time of sampling. With the exception that the time relationship for the cross-over was later at 68° F. than in previous studies at 85° F. the general relationship is that which has already been established and discussed (Wardlaw and Leonard, 1940). Lastly, the curve D of Fig. 8 shows the changing pulp/skin weight ratio during the course of the experiment, increasing values being observed from the onset of the climacteric until final senescence.

The fruits on which these respiration studies were made have been used, after the determination of the CO_2 content, for an analysis of the principal carbohydrate metabolites; they thus afford interesting material for a study of the precise time relationship between the respiration changes recorded and the changes undergone by these metabolites. The results will be considered in a later communication.

It has already been noted that the fruits used in the above experiment were derived from the same bunch as those used for the experiments described in section II. Comparison of the curves and ripening changes, however, shows that the ripening of the latter was considerably delayed. The major difference in treatments was that the fruits used in section II were placed at 53° F. within 4 hours of harvesting, but those considered in the present section were held at 85° F. for 33 hours. Attention has already been directed to the fact that the period immediately following harvesting is a critical one in respect of metabolic change, a view which is substantiated by the data presented here. Further exploration of this phase, which is of very considerable physiological interest and practical importance, will call for an extensive experimental programme which is envisaged as an essential link in these studies. In view of the disparity of ripening rates indicated above no attempt is made at this stage to define the correlations between internal gas concentrations and respiration rate; this will be treated later in sections IV and V.

While, relative to the striking changes taking place at the climacteric, the preclimacteric trends in internal gas concentrations at 53° F. and 68° F. (Fig. 4) show only small changes, nevertheless these are consistently in the direction of increasing concentration of CO_2 as indicating a changing rate of metabolism, this being borne out by chemical analyses of the carbohydrate metabolites. In other words, while storage at lower temperatures slows down the ripening changes, it is essentially a protraction of these processes and may in certain circumstances produce deviations from the normal trend. On this aspect the comprehensive respiration data submitted here may be expected to throw new light.

(c) *At 53° F. throughout.*

Fig. 9 gives the record of internal concentrations of CO_2, O_2, and CO_2 *plus* O_2 in a fruit held at 85° F. for 33 hours and thereafter at 53° F. and 85 per cent. R.H. throughout,[1] this being generally typical of the several records

[1] For temperature and humidity records see Fig. 23.
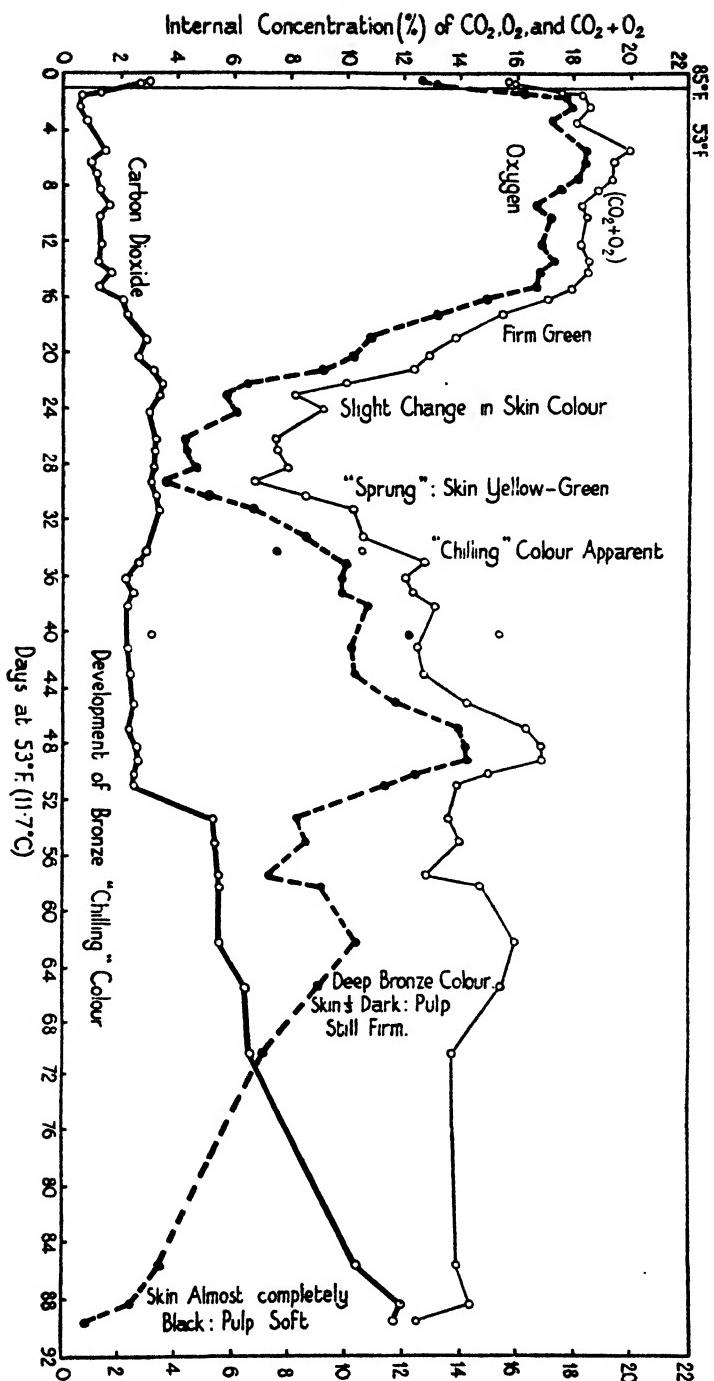
FIG. 9. Percentage internal concentrations of CO_2, O_2, and $(CO_2 + O_2)$ for a '½-full' banana at 85° F. for a preliminary period followed by storage at 53° F. and 85 per cent. R.H. continuously.

obtained from this series of fruits. The changes in internal concentrations on transference from 85° F. to 53° F., and thereafter the steady trends during the first 15 days, have already been discussed. Considerable changes in the trends of the curves began to be apparent on the 15th–16th day for the individual fruit illustrated in Fig. 9, but reference to Fig. 10[1] shows that for the several
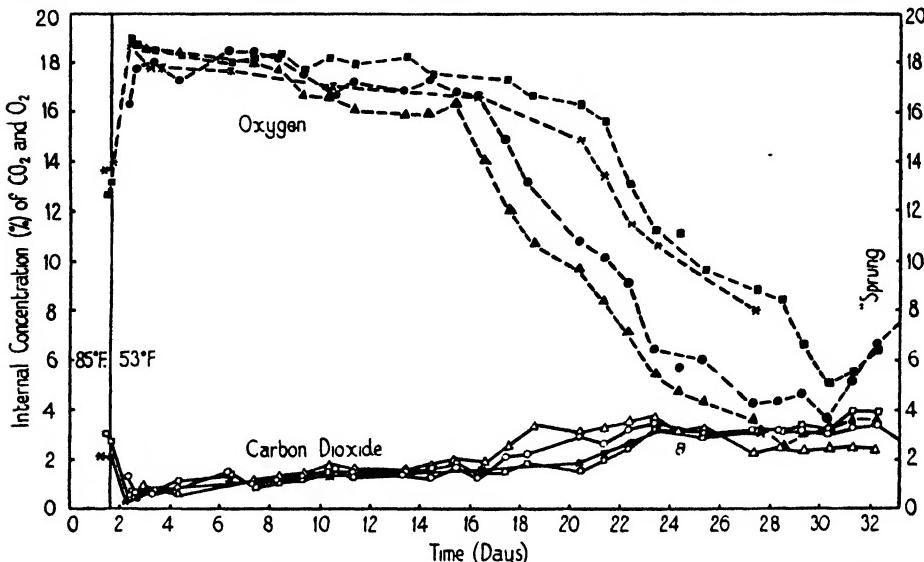
FIG. 10. Percentage internal concentrations of CO_2 and O_2 for four '⅓-full' bananas from the same hand as Fig. 9 during continuous storage at 53° F. and 85 per cent. R.H. after an initial period at 85° F. The marked effect on the internal gas concentrations on transferring fruit from 85° to 53° F. is clearly shown. The time spread in the onset of ripening is clearly shown by the distribution of the curves of internal O_2 concentration.

fruits under observation, such changes in trend were spread over a period reaching up to the 22nd day. In both Figs. 9 and 10 it will be seen that the rapid decline in internal O_2 concentration from the previous gradual downward trend took place at a value of approximately 16 per cent. The possible implications in the initiation of ripening of the part played by internal O_2 diminishing to a critical concentration have already been considered, and would appear to be further substantiated by the data set out here. At 85° F. the apparent critical oxygen concentration prior to the climacteric rise is lower than at the lower temperatures considered here.

As at higher temperatures, the onset of ripening (Fig. 9) is marked by a rapid fall in the internal concentration of O_2 and a rise in that of CO_2. But whereas the O_2 curve is comparable with those obtained at higher temperatures in that well-defined minimal values were obtained, the CO_2 concentration curve rose to a maximum of not more than 4 per cent. and, further, did not show a typical climacteric peak but rather a sustained level. As a result of these trends the curve of CO_2 *plus* O_2 shows a very well-marked trough during

[1] See footnote on p. 390.

this phase. The annotations in Fig. 9 indicate the progress of ripening, the 'sprung' condition following immediately on the attainment of the minimum O_2 concentration, an observation which is confirmed by other records obtained at this temperature. Following on this phase there was a slow downward trend
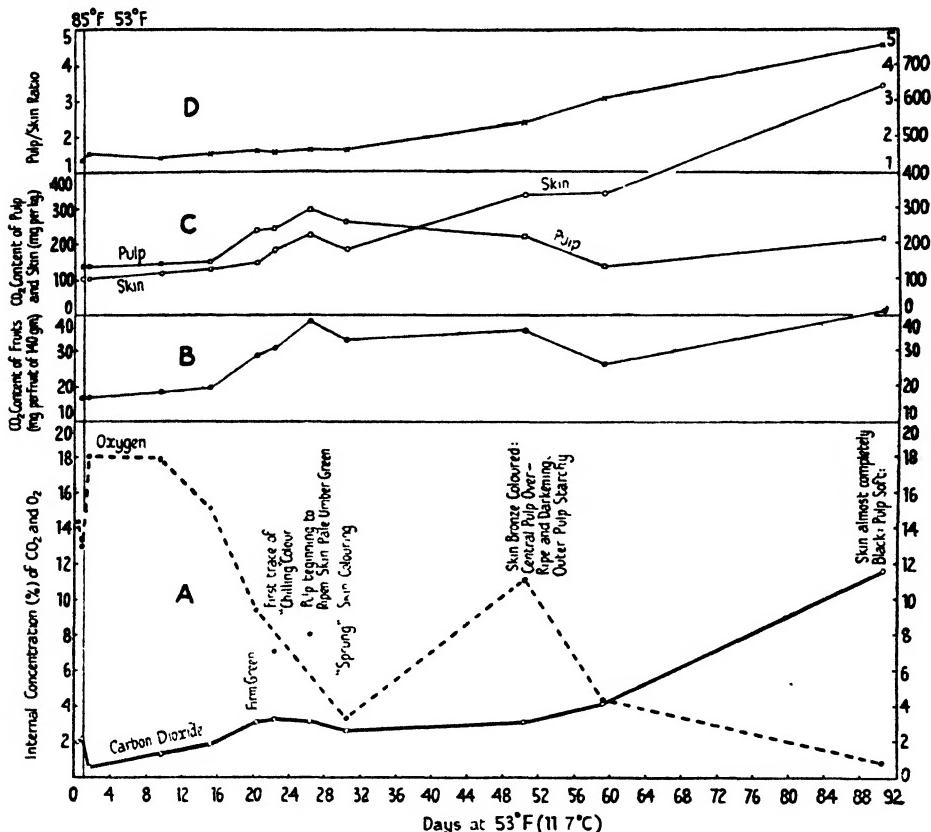
FIG. 11. Composite curves (see text) for '¾-full' bananas at 85° F. for a preliminary period followed by continuous storage at 53° F. and 85 per cent. R.H. A. Percentage internal concentrations of CO_2 and O_2. B. CO_2 content of fruit. C. CO_2 content of pulp and skin. D. Pulp/Skin weight ratios.

in the curve of internal CO_2 concentration as in the curve of respiration rate (Fig. 2), and a slow but well-defined recovery in O_2 concentration to 15 per cent. over 20 days (29th to 49th). During this period the development of 'chilling' colours (Wardlaw and McGuire, 1931) in the skin and in the inner pulp, of other fruits of the same series (Fig. 11),[1] was a conspicuous feature. The last 40 days (49th to 89th) of the senescent phase were marked by a downward trend towards the extinction point in the concentration of O_2 as in ripening at higher temperatures, and by a slow rise to high values in the

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[1] Temperature and humidity records of Fig. 23.

internal concentration of CO_2, e.g. 12 per cent. as compared with 4 per cent. at the climacteric. (The sharper rise in concentration of CO_2 on the 52nd day observed in this fruit may be indicative of a definitive metabolic change, but was not equally apparent in other fruits of this series; it coincided with a rise and fall in the room temperature, Fig. 23.) In Fig. 2 it will be seen that the senescent phase is characterized by a steady respiration rate.

In Fig. 11 composite curves of the CO_2 content of whole fruits, and of skin and pulp, and pulp/skin weight ratios are given, together with the trends in the internal concentrations of CO_2 and O_2 for this series of fruits. The major changes in the curves of the internal concentrations of CO_2 and O_2 (A of Fig. 11) are in close agreement, both in respect of time and values, with the record for the single fruit shown in Fig. 9. As in previous records of this type, the CO_2 content of the whole fruit (B of Fig. 11) follows closely the general trend of the curve of internal concentration. The relationship between the CO_2 contents of pulp and skin during ripening at 53° F., Fig. 11 (C), is closely comparable with that obtained for fruit ripening at 68° F. (C of Fig. 8), a crossing over of the curves during the senescent phase again being noted. The curve of pulp/skin weight ratio (D of Fig. 11) is also typical, high values being reached during final senescence.

During the preparation for the estimation of the CO_2 content of the fruits used for the data of Fig. 11, iodine tests for starch were made. These showed that starch disappeared very slowly though finally only traces remained, and that such disappearance took place originally from the central placental region outwards to the skin, the central strand, however, retaining its starch content apparently unaltered. Estimations of the carbohydrate metabolites of these fruits are now available and will be considered in a subsequent paper.

IV. RESPIRATION OF 'HEAVY ¾-FULL' FRUIT AT 53° F. AND RIPENED AT 65° F.

(a) *Materials and methods.*

In the experiments described in this section and section V the underlying purpose was to obtain comprehensive respiration data on 'heavy ¾-full' fruit as compared with '¾-full'; and, in keeping with commercial practice with such fruit, the storage period at 53° F. was reduced from 15 to 11 days, ripening being undertaken at 65° F. instead of 68° F. It is known that the more mature the fruit at the time of harvesting the shorter is the period before ripening changes occur. But as already indicated (Wardlaw, Leonard, and Barnell, 1939), size of fruit does not provide an invariable guide to its physiological maturity. Occasionally consignments of fruit have been found to possess a maturity considerably less than that anticipated on the basis of size (Wardlaw and Leonard, 1940). The fruit to be considered in the present section falls into this category. It may be regarded as typical of certain heavy grades of fruit produced throughout the Caribbean on good soils under favourable

conditions of rainfall, where bunches tend to be of high 'count', i.e. 10 to 11 hands.

It has already been established that during ripening at tropical temperatures there is a very close relationship between the curve of respiration rate and that for the internal concentration of carbon dioxide. It was important therefore to ascertain to what extent this relationship was maintained in fruit ripening at lower temperatures.

In order to establish the exact time-relationship between internal gas concentrations and respiration rate fruits were fitted with sampling tubes and placed in respiration chambers as already described (Wardlaw and Leonard, 1939). Since this arrangement precludes the determination of transpiration by loss in weight, this was obtained from other fingers exposed in the storage room. The fruit was harvested during the morning, received at the research station at 1.0 p.m., and the experiment at 53° F. begun by 2.30 p.m., i.e. not more than six hours from cutting.

(b) *Respiration rate and internal concentrations of CO_2 and O_2.*

Figs. 12 and 13 show the respiration rate and the internal concentrations of CO_2, O_2, and CO_2 *plus* O_2 of two 'heavy $\frac{3}{4}$-full' fruits held at 53° F. for 11 days and thereafter at 66° F., the air in the respiration chamber being at saturation throughout. Values for the temperature of the air in the respiration chamber and, at intervals, the carbon dioxide concentration are also given.

During the period at 53° F., steady levels were maintained in the respiration rate and internal concentrations of carbon dioxide and oxygen. Transition to 66° F. was marked by a rapid rise in respiration rate followed by a decline, by a rise to a new level in the internal concentration of CO_2, and by a readjustment to a lower level of the internal concentration of O_2. A well-established level in respiration rate and internal gas concentrations then obtained at 66° F. prior to the climacteric. As mentioned in section IV (*a*) above, the onset of the climacteric was delayed abnormally for the grade of fruit employed. Climacteric changes were in conformity with those previously described for '$\frac{3}{4}$-full' fruit at 69° F. The close approximation in time between the attainment of minimal internal O_2 concentration and maximal internal CO_2 concentration and respiration rate is clearly shown in both Figs. 12 and 13.

The after-peak phase is characterized by a recovery in the internal concentration of O_2 to high values, and by a preliminary decrease in respiration rate and internal concentration of CO_2, followed by a very considerable period of steady values. Up to approximately the end of the 'eating-ripe' stage, the very close parallelism between the curves of respiration rate and internal CO_2 concentration was maintained. Thereafter evidence of increasing tissue resistance to gaseous movement was seen in the rising internal concentration of CO_2 accompanied by a decline in the respiration rate. During this period the curve of internal concentration of O_2 showed a steady decline. Final senescence was characterized by a declining respiration rate, by a considerable increase in

D d
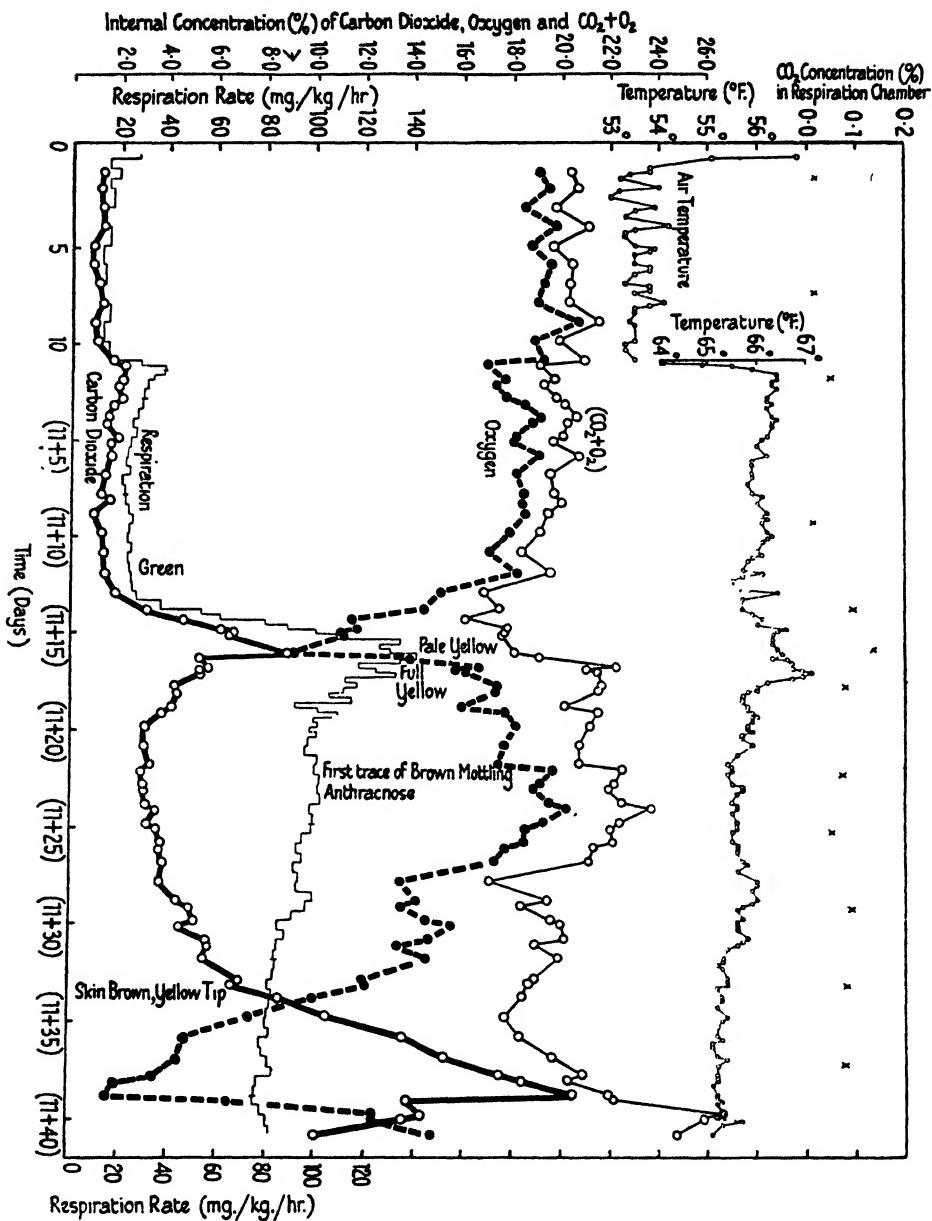
FIG. 12. Respiration rates and percentage internal concentrations of CO_2, O_2, and (CO_2 + O_2) for a 'heavy $\frac{3}{4}$-full' banana at 53° F. (approx.) and 100 per cent. R.H. for 11 days followed by ripening at 66° F. (approx.). Temperature and CO_2 concentrations in the respiration chamber air as in Fig. 1. (See text.)

the internal concentration of CO_2, and by low, followed by increasing, internal concentrations of O_2. A commentary on these trends has been given in previous sections and in a previous paper (Wardlaw and Leonard, 1940).
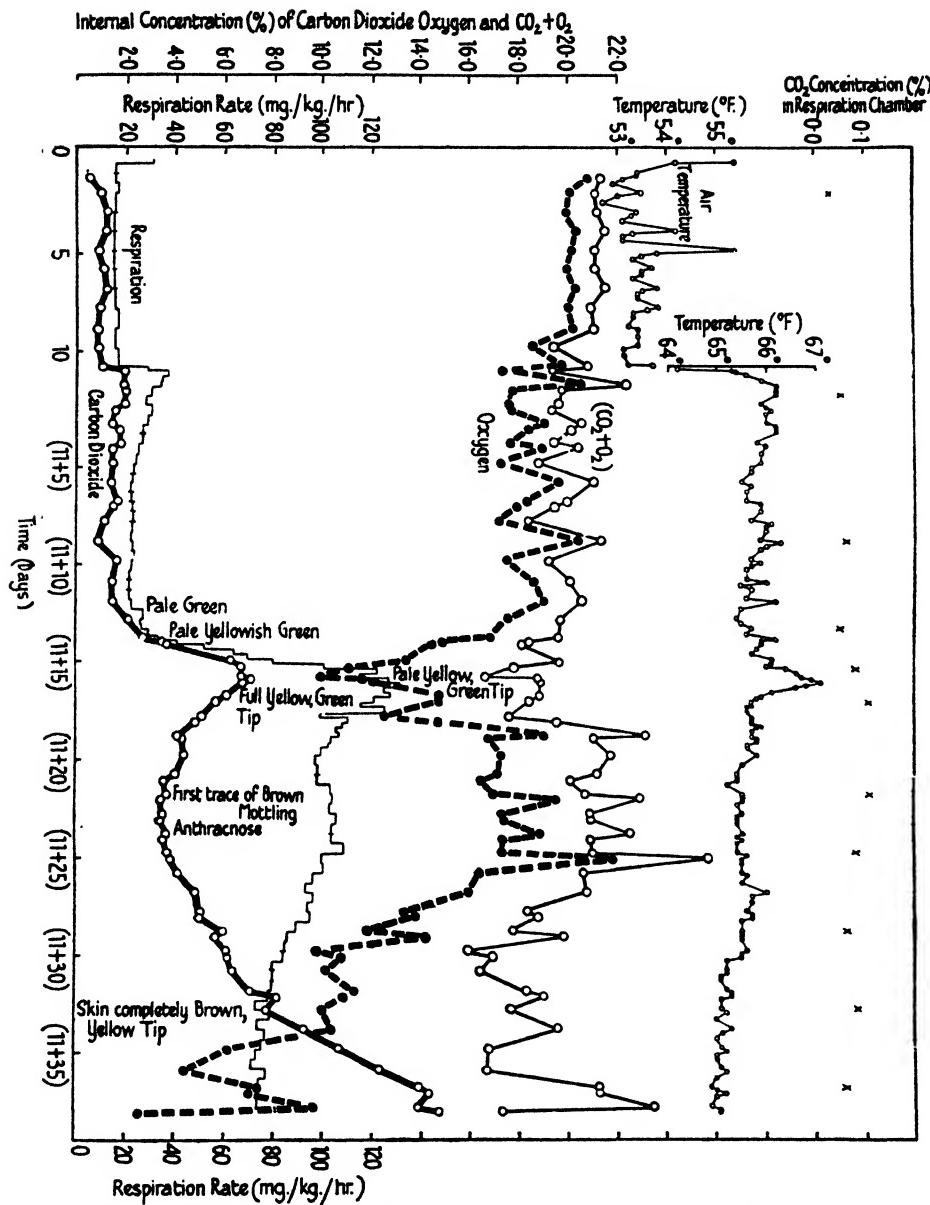
{FIG. 13. Similar data to those of Fig. 12, but for another comparable fruit.

The very close general similarity between the two fruits of Figs. 12 and 13 is apparent. Nevertheless, small individual differences, e.g. in time of onset of the climacteric, are perceptible. This uniformity of material with its minor variations has already been discussed. The final senescent phase indicated in Fig. 12 presents some points of special interest. During the last three days

of the experiment, when the fruit was completely brown, soft, watery, and covered by fungal mould, it will be noted that whereas the curve of respiration rate remained practically unchanged the curve of internal concentration of CO_2 fell rapidly from its high value, while that of internal concentration of O_2 rose rapidly from its low value. Without entering on a discussion of the several implications of such records, it may be mentioned that fruits at this stage of senescence have been observed to show a tendency to part along the sutures—possibly the equivalent of dehiscence—a phenomenon which would in part account for the changes in internal gas concentrations observed.

The progress of ripening change is shown in Figs. 12 and 13; important data are cited in Table III:

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TABLE III

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	Fruit 3 C (Fig. 12).	Fruit 1 C (Fig. 13).	
Initial weight of fruit	186·4	172·2	gm.
Respiration rate at 53° F. . . .	14	15	mg./kg./hr.
Mean internal gas concentration at 53° F.: CO_2	1·0	1·0	per cent.
,, ,, ,, ,, ,, O_2	19·0	20·0	,,
Respiration rate at 66° F. (preclimacteric level)	23	22	mg./kg./hr.
Mean internal gas concentration (preclimacteric level) at 66° F.: CO_2	1·3	1·5	per cent.
,, ,, ,, ,, O_2	18·4	18·8	,,
Respiration rate at climacteric peak . .	142	130	mg./kg./hr.
Internal gas concentrations at climacteric peak: CO_2	7·2	6·8	per cent.
,, ,, ,, ,, O_2	8·9	10·6	,,
Respiration rate during post-climacteric (steady level)	100	100	mg./kg./hr.
Mean internal gas concentrations during post-climacteric: CO_2	3·0	3·5	per cent.
,, ,, ,, ,, O_2	18·8	17·2	,,
Respiration rate at final senescence . .	74	74	mg./kg./hr.
Internal gas concentrations at final senescence:			
Maximal CO_2	20·4	14·0	per cent.
Minimal O_2	1·6	4·4	,,
Final weight of fruit	167·5	156·9	gm.
Loss in weight (% of initial weight) . .	10·16	8·87	per cent.
Pulp/Skin weight ratio	2·47*	3·32	

<div align="center">

* low value: pulp attached to skin.

</div>

(c) *Internal CO_2 concentration and content of tissue.*

Additional observations on internal gas concentrations were made on a number of fruits from the same bunch as in section IV (*b*) above, the fruits being exposed on the bench at 53° F. and 85 per cent. R.H. for 11 days and thereafter at 65° F. and 85 per cent. R.H.[1] A typical record for a single fruit is shown in Fig. 14. During the period at 53° F. steady levels of internal concentration of CO_2 and O_2 were maintained, but by comparison with Figs. 12

[1] Temperature and humidity records at 53° F. for days 51·5 to 62·5 are shown in Fig. 25, at 65° F. in Fig. 26.
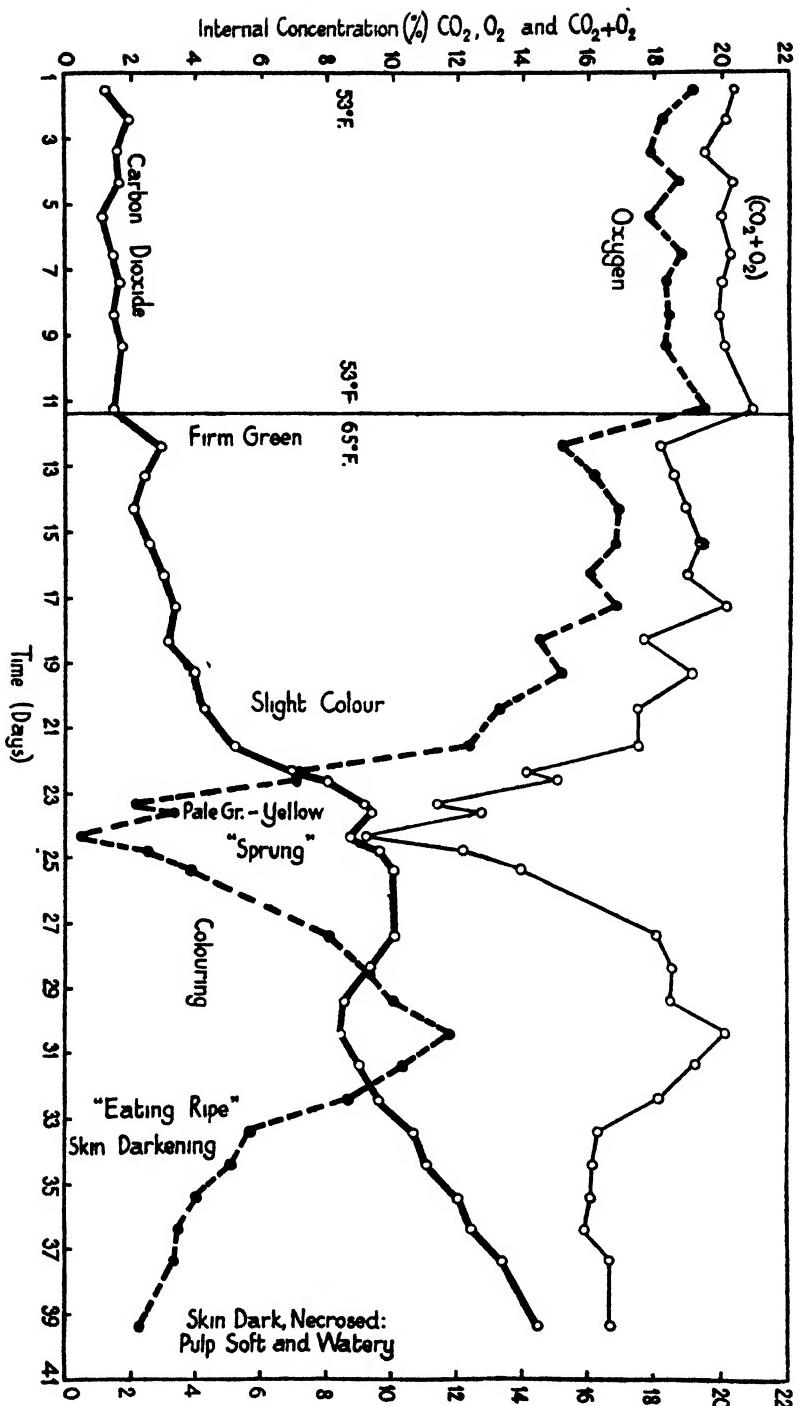
FIG. 14. Percentage internal concentrations of CO_2, O_2, and (CO_2+O_2) for a 'heavy ¾-full' banana at 53° F. for 11 days with subsequent ripening at 65° F. and 85 per cent. R.H. Compare with Figs. 12 and 13 at 100 per cent. R.H.

and 13 it will be noted that the internal concentration of CO_2 was consistently higher and that of O_2 consistently lower—a reflection of the response to differences in the environment, i.e. 85 per cent. R.H., Fig. 14, as compared with 100 per cent. R.H. in Fig. 12 (compare also tabulated data).

Transference to 65° F. resulted in a well-marked rise in the internal concentration of CO_2 and an even more marked decline in O_2 concentration, each showing a slight subsequent recovery to a fresh level, which, however, was of brief duration, being followed by the onset of climacteric changes. With the qualification that at the climacteric higher CO_2 and lower O_2 values than those shown in Figs. 12 and 13 were obtained, the trends shown in Fig. 14 are generally comparable, i.e. the CO_2 curve rises to a broad hump, declines slightly, then rises during final senescence; the O_2 curve shows the typical climacteric trend to low values, a recovery to a higher values, and a subsequent decline towards the extinction point during late senescence.

The following data of Fig. 14 are cited:

TABLE IV

Initial weight of fruit		197·8	gm.
Mean internal concentration of gases at 53° F.:	CO_2	1·6	per cent.
,, ,, ,, ,,	O_2	18·4	,,
Mean internal concentration of gases at 65° F. (preclimacteric):	CO_2	2·4	,,
,, ,, ,, ,,	O_2	16·8	,,
Internal concentration of gases at climacteric peak:	CO_2	10·0	,,
,, ,, ,, ,,	O_2	0·5	,,
Internal concentration of gases at post-climacteric peak:	CO_2	8·4	,,
,, ,, ,, ,,	O_2	11·0	,,
Internal concentration of gases (final senescence):	CO_2	14·5	,,
,, ,, ,, ,,	O_2	2·2	,,
Final weight of fruit		158·2	gm.
Loss in weight (% of initial weight)		20	per cent.
Pulp/Skin weight ratio		3·69	

The differences already noted in the internal gas concentrations in fruit at 100 per cent. and 85 per cent. R.H. during storage at 53° F. became still more accentuated during ripening at 65° F. A comparison of the internal concentrations of CO_2 and O_2 for the fruits of Fig. 13 and Fig. 14 shows differences as follows: (i) climacteric changes were initiated earlier in the fruit at 85 per cent. R.H.; (ii) peak values for the internal concentration of CO_2 were higher and O_2 values considerably lower in the fruit at 85 per cent. R.H., this relationship being maintained during senescence; (iii) the duration of the senescent period was considerably more extended in the fruit at 100 per cent. R.H., i.e. 8 days. The importance of water losses in increasing the resistance which tissues offer to the movement of gases has been discussed elsewhere (Wardlaw and Leonard, 1940), and is again exemplified by the data submitted here.

It may also be noted at this point that the trends of the curves of internal concentrations of CO_2 and O_2 given by this series of fruits are in general agreement with those for '$\frac{3}{4}$-full' fruit at 53° F. followed by ripening at 68° F., and with '$\frac{3}{4}$-full' and 'full' fruit ripening at tropical temperatures.

As in previous experiments a series of fruits was selected for the determination of internal gas concentrations and CO_2 content of tissue; the results are given in the composite diagram Fig. 15. In Fig. 15, curve A, it will be observed that the general trends of internal concentrations of CO_2, O_2, and CO_2 *plus*
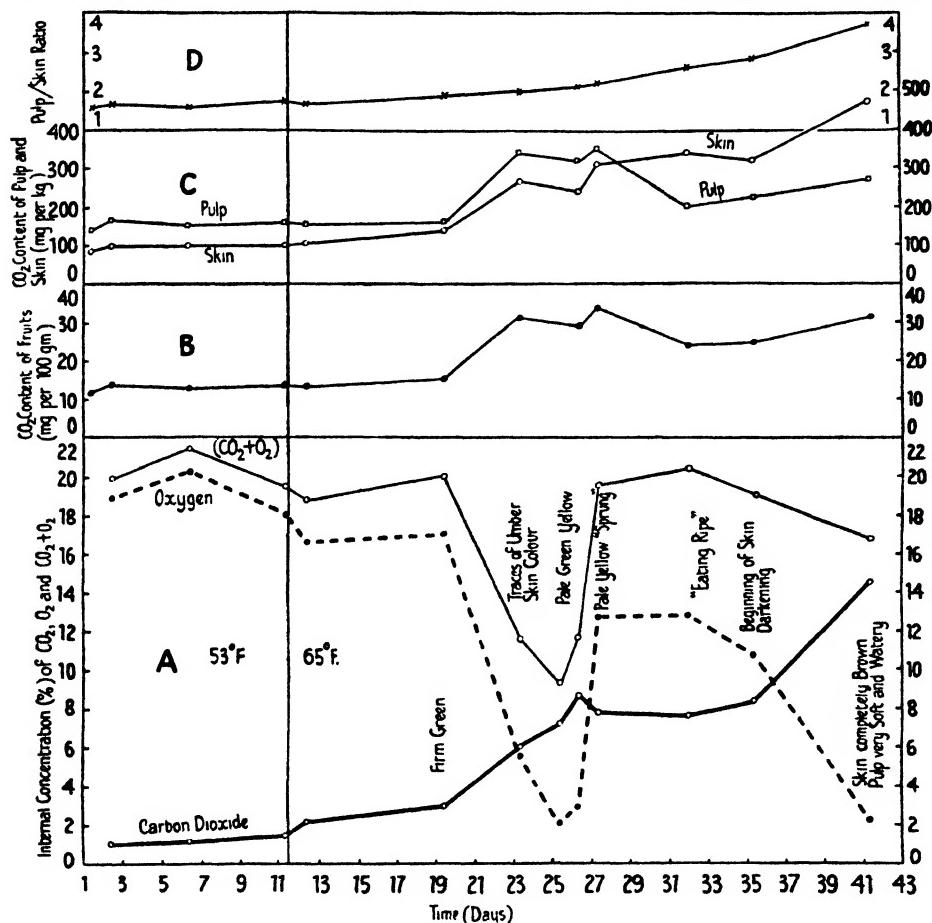
FIG. 15. Composite curves (see text) for 'heavy $\frac{3}{4}$-full' bananas at 53° F. and 85 per cent. R.H. for 11 days with subsequent ripening at 65° F. and 85 per cent. R.H. A. Percentage internal concentrations of CO_2, O_2, and (CO_2+O_2). B. CO_2 content of fruit. C. CO_2 content of pulp and skin. D. Pulp/Skin weight ratios.

O_2 obtained from the series of fruit are very similar to those given in Fig. 14 for a single fruit of the series. The stages of ripening are annotated. In Fig. 15, curve B, which gives the CO_2 content of the whole fruit, the general parallelism with the curve of internal CO_2 concentration will again be noted, high content being conspicuous during the climacteric and final senescent phases. Fig. 15, curve C, shows the CO_2 content of pulp and skin, and Fig. 15, curve D, the pulp/skin weight ratio, both of which exemplify the trends already established and discussed previously.
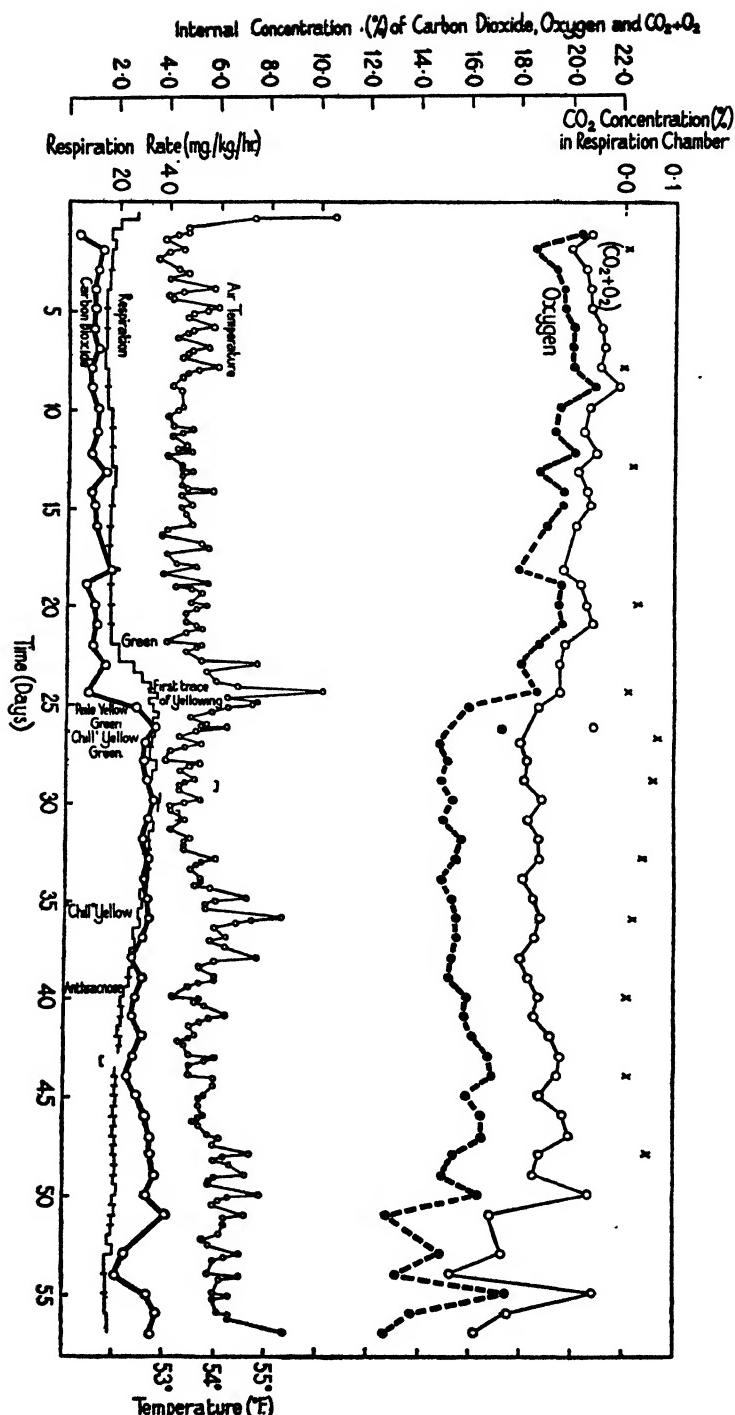
FIG. 16. Respiration rate and percentage internal concentrations of CO_2, O_2, and (CO_2+O_2) for a 'heavy ¾-full' banana at 53° F. (approx.) and 100 per cent. R.H. continuously. Temperature and CO_2 concentration of respiration chamber air as in Fig. 1.

V. Respiration of 'Heavy $\frac{3}{4}$-Full' Fruit at 53° F. throughout

(a) *Respiration rate and internal concentrations of CO_2 and O_2.*

The methods and materials were those described in section IV (a) above, the fruits being held at 53° F. and 100 per cent. R.H. throughout the period of the experiment.

Fig. 16 gives the respiration rate and internal concentrations of CO_2, O_2, and CO_2 *plus* O_2 during an experimental period of 57 days, when the experiment was arbitrarily concluded. The respiration rate maintained a steady level for the first 22 days, and rose to a higher level, i.e. the climacteric, during 3 days, whereafter there was a slow but steady fall until the 44th day; this was followed by a steady level for 10 days and then by a further slight decline. A companion fruit (Fig. 17) showed a steady decline in respiration rate from the climacteric onwards.

The following citation of data is submitted:

Table V

		Fruit 2 C (Fig. 16).	Fruit 4 C (Fig. 17).	
Initial weight of fruit		166·1	182·9	gm.
Respiration rate during preclimacteric . .		17	17	mg./kg./hr.
Internal gas concentration:	CO_2	1·2	1·2–2·4	per cent.
,, ,, ,,	O_2	20·1	20·0–18·0	,,
Respiration rate at climacteric peak . .		36	34	mg./kg./hr.
Internal gas concentration: Maximum	CO_2	3·6	5·5	per cent.
,, ,, ,, Minimum	O_2	14·8	4·8	,,
Respiration rate post-climacteric (44th day) .		20	18	mg./kg./hr.
Internal gas concentration:	CO_2	2·6	3·2	per cent.
,, ,, ,,	O_2	16·8	13·4	,,
Respiration rate at conclusion of experiment (57th day)		20	14	mg./kg./hr.
Internal gas concentration:	CO_2	3·5	3·8	per cent.
,, ,, ,,	O_2	12·7	11·4	,,
Time to reach onset of climacteric . .		22	20	days
Final weight of fruit		157·0	173·7	gm.
Loss in weight (% of initial weight) . .		5·46	5·02	per cent.
Pulp/Skin weight ratio		3·08	2·91	

The general parallelism between the curves of respiration rate and internal concentration of CO_2 during the preclimacteric and climacteric phases is again demonstrated. During the post-climacteric phase, with the further development of ripening changes in the tissues, increased resistance to the movement of gases takes place and the falling respiration rate is seen to be accompanied by an increase in the internal concentration of CO_2.

The close similarity between adjacent fingers from the same row has on several occasions been noted in these papers. Nevertheless quite considerable differences in weight may be observed. As the fruits are of approximately the same length such differences in weight must be reflected in different

surface/volume relationships, which will in turn affect the several aspects of gaseous interchange involved in respiration. In brief, it will be noted that in the heavier fruit (Fig. 17) there was a consistently lower respiration rate, higher internal concentration of CO_2, and lower internal concentration of O_2 during the steady pre- and post-climacteric periods and more marked trends at the climacteric.

By comparison of Figs. 12 and 13, and Figs. 16 and 17, it will be seen that the time of onset of the climacteric at the two temperatures, i.e. 66° and 53° F., was approximately the same. Apart from again directing attention to the notes on the nature of these fruits, section IV (a), no comprehensive explanation of the behaviour can be offered. It may perhaps be noted, however, that in the fruits used in this experiment a low resistance to the movement of gases was a prominent feature during the preclimacteric phase. In such fruit substances produced by the tissues known to stimulate ripening, such as ethylene, would tend to escape readily into the atmosphere and thus not accumulate to the threshold value for initiation of the ripening processes (Gane, 1936). It will also be noted *a propos* of the previous discussion of the role of diminishing O_2 concentrations in the initiation of ripening (Wardlaw and Leonard, 1940) that in these fruits high internal concentrations of this gas persisted at both temperatures prior to the climacteric.

(b) *Internal CO_2 concentration and content of tissue.*

Additional observations on internal gas concentrations were made on a number of fruits from the same bunch as in section V (a) above, the fruits being exposed on the bench at 53° F. and 85 per cent. R.H.[1] A typical record of the internal gas concentrations for a single fruit is shown in Fig. 18. It will be noted that the minimum O_2 concentration recorded at the climacteric, as indicated by the curve of CO_2 concentration, occurred at a considerably later stage than is typical for fruits ripening at higher temperatures. In this fruit, as also in the '¾-full' fruit ripening at 53° F., the attainment of the 'sprung' condition followed almost immediately on the attainment of minimal O_2 concentrations. The experiment recorded in Fig. 18 ended on the 54th day at which stage the CO_2 concentration curve was beginning to show the typical rise associated with the final senescent phase, while the curve of internal O_2 concentration was showing a corresponding decline. Nevertheless, although the skin had acquired the deep bronze colour associated with chilling with some darkened areas, the pulp of this fruit was 'sprung' but still comparatively firm, and the placental pulp still contained a small amount of starch with a gradation to a medium starch content in the outer pulp. The fruit was not judged to have reached the 'eating ripe' stage though regional ripening in the placentae and the presence of 'chill' darkening in that region was apparent. It will be recalled (Figs. 8 and 9) that '¾-full' fruits were kept under observation at 53° F. up to 90 and 105 days, at which stage the skin was of a parch-

[1] Temperature and humidity records in Fig. 23, 51·5 days onwards.
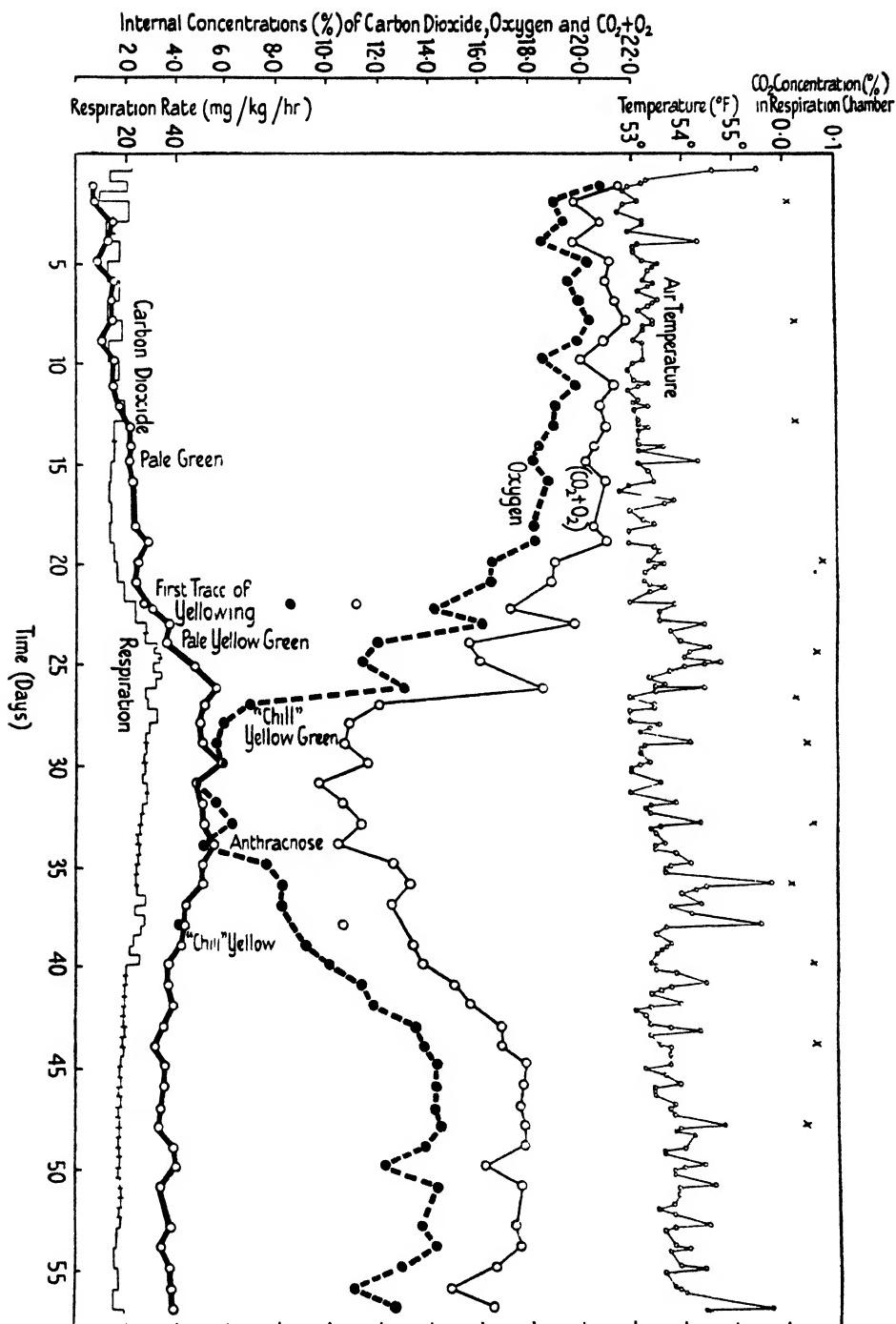
FIG. 17. Similar data to those of Fig. 16, but of another comparable fruit. A comparison of Figs. 16 and 17 particularly in respect of the internal concentrations of CO₂ and O₂ indicates the differences that may exist in closely comparable fruits from the same hand (see text).

ment-like thinness, dark and necrotic, while the pulp was exceedingly soft and watery and almost completely devoid of starch.

By comparing Fig. 18 with Fig. 9 ('¾-full' fruit continuously at 53° F.) it will be noted that in the latter the curve of internal O_2 concentration not only
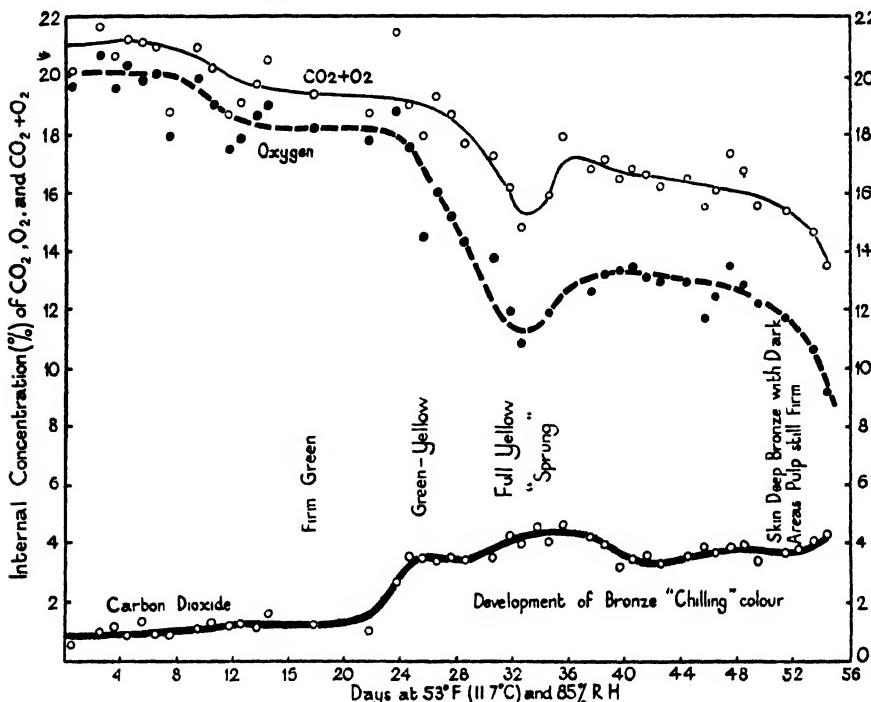
FIG. 18. Percentage internal concentrations of CO_2, O_2, and (CO_2+O_2) for an upper row 'heavy ¾-full' banana at 53° F. (approx.) and 85 per cent. R.H. continuously. Compare Fig. 19.

shows lower values in general, but also shows very much more pronounced trends during the several ripening phases. The comparatively less well-marked trends in Fig. 18 and the maintenance of higher values of oxygen concentration may be attributed to the higher porosity already associated with this category of fruit. The trends and values of internal carbon dioxide concentration, on the other hand, are closely comparable.

As in Fig. 9 so in Fig. 18 it will be seen that the minimal internal concentration of oxygen at the climacteric did not occur until the new, higher level of internal carbon dioxide concentration had already been established for some considerable time, i.e. 8 days. Again, the 'sprung' condition, as before, followed almost immediately on the attainment of minimal oxygen concentration.

For comparison with Fig. 18 the internal gas concentrations for a lower row fruit from the same hand are shown in Fig. 19. Here it will be noted that the curve of internal concentration of CO_2 shows a rise and fall at the climacteric

instead of a rise to a new level as in Fig. 18, while the curve of internal oxygen concentration shows generally lower values and a more marked trough at the climacteric (c). Such comparisons direct attention to the importance of structure and gaseous exchanges in respiration between an approximately straight, upper row fruit, Fig. 18, and a curved, lower row fruit, Fig. 19.

Again, comparing Fig. 18 with Fig. 14, i.e. for corresponding fruit ripening
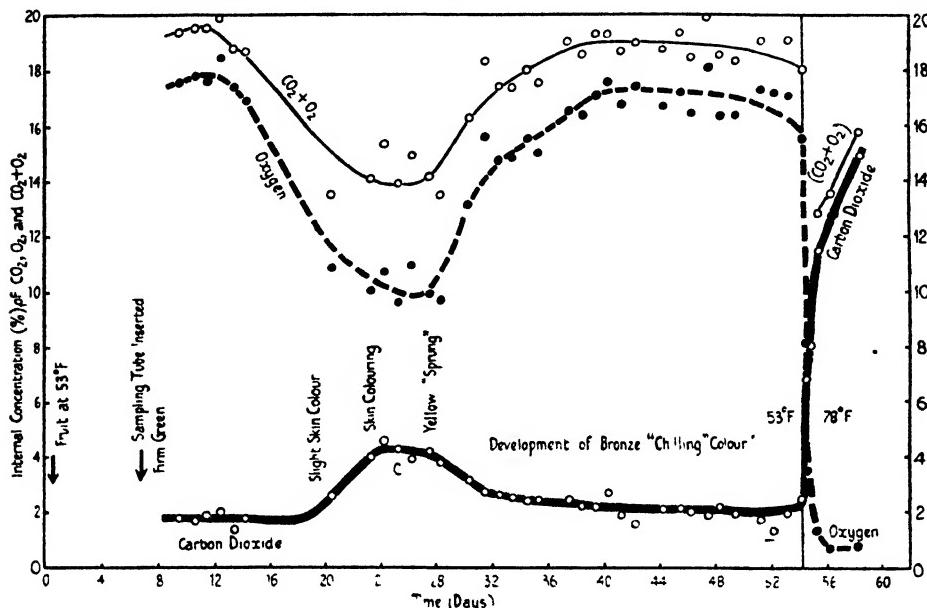
FIG. 19. Percentage internal concentrations of CO_2, O_2, and (CO_2+O_2) for a lower row finger from the same hand as Fig. 18 at 53° F. (approx.) and 85 per cent. R.H. for 54 days. Compare Fig. 18. Note the very rapid rise in the curve of internal concentration of CO_2 and fall in that of O_2 when the fruit was transferred from 53° F. to 78° F. on the 54th day. C = Climacteric peak.

at 65° F., it will be noted that the time of incidence of the climacteric was approximately the same at the two temperatures, i.e. peak was at the 23rd–25th day. The greater water loss sustained by the fruit at 65° F. and the increased rate of metabolism are reflected in the considerably more marked trends in the curves of internal concentration of CO_2 and O_2.

Fig. 20 gives the data obtained from a series of fruits selected for the determination of the internal concentration and tissue content of carbon dioxide. It will be seen by comparison with Fig. 18 that the general trends of internal concentrations of CO_2, O_2, and CO_2 *plus* O_2 (Fig. 20, curve A) are in general agreement with those for a single fruit. In Fig. 20, curves B and C, the trends of the CO_2 content of tissues during ripening at 53° F. are in agreement with those already illustrated for '¾-full' fruit (Fig. 11, curves B and C) while a fairly general agreement is seen to exist in actual values. In Fig. 20, curve D, by comparison with Fig. 11, curve D, it will be seen that the effect of the heavier
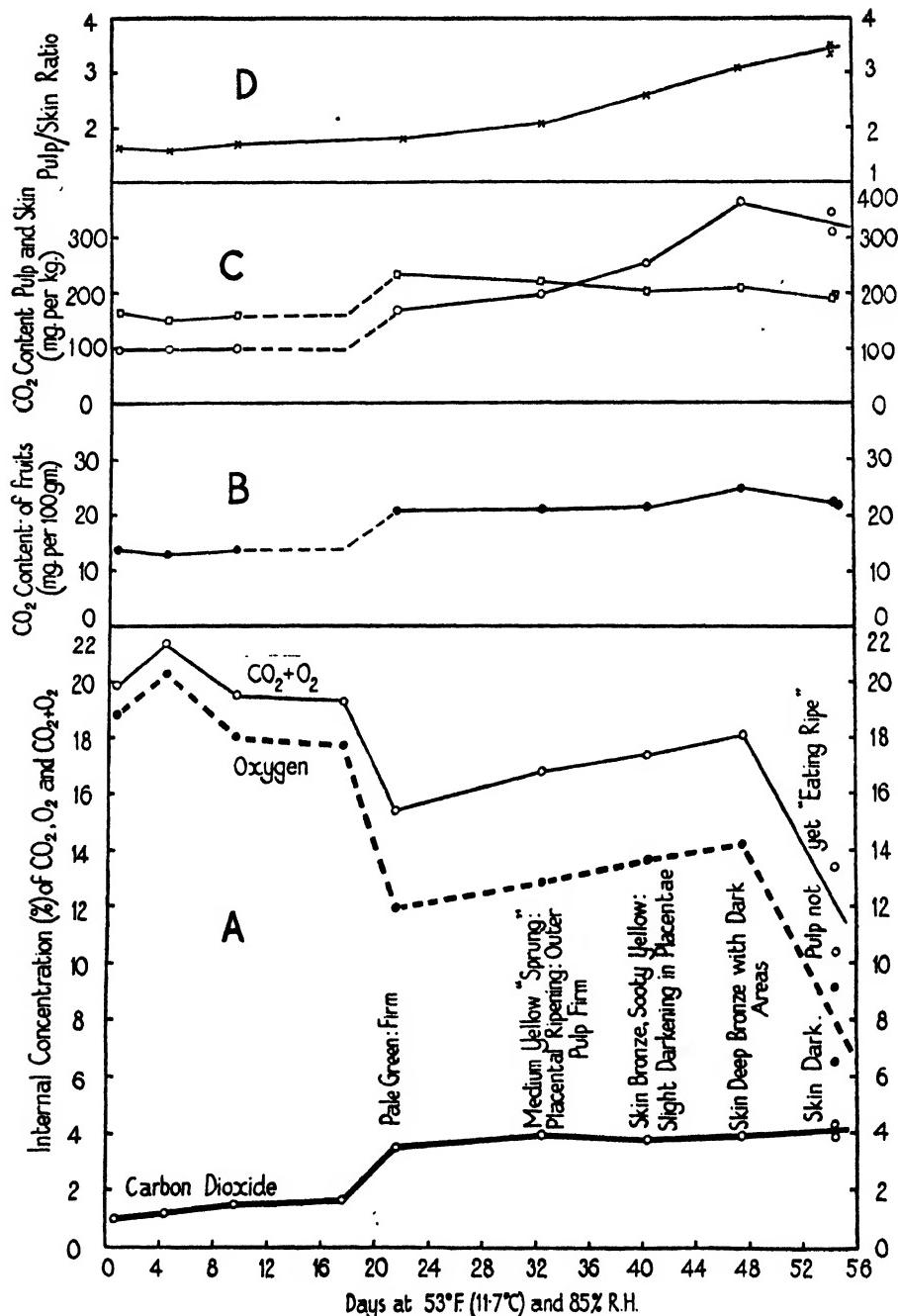
FIG. 20. Composite curves (see text) for 'heavy ½-full' bananas at 53° F. (approx.) and 85 per cent. R.H.　A. Percentage internal concentrations of CO_2, O_2, and (CO_2+O_2).　B. CO_2 content of fruit.　c. CO_2 content of pulp and skin.　D. Pulp/Skin weight ratios.

grade of fruit used is reflected in higher pulp/skin weight ratios (Wardlaw, Leonard, and Barnell, 1939). In the series of fruits of Fig. 20, of which Fig. 18 gives an individual record, it has been consistently noted that for this category and type of fruit the onset of ripening-change was denoted in the curve of internal oxygen concentration by well-marked turning, i.e. threshold values at approximately 18 per cent. This is also shown in Fig. 19 for a lower row fruit from the same hand.

The principal carbohydrate metabolites of the fruits used in Fig. 20 have been estimated: the results are available for comparison with the other series mentioned in the foregoing sections.

(c) *Internal gas concentrations in a fruit developing fungal disease.*

With rare exceptions the observations on internal gas concentrations carried out in the course of this work have been maintained over considerable periods without interference due to accidental fungal contamination. Accordingly, the several curves submitted in this and previous papers may be accepted as approximately representative of the internal gas composition of fruits during the several phases of normal ripening. Where fungal contamination has accidentally resulted from the insertion of the gas-sampling tube very different, and recognizably aberrant, trends become evident. Fig. 21 illustrates a case in point where a fruit from the same hand as that used above showed evidence of infection after 9 days at 53° F., whereupon in the course of a further 9 days the internal concentration of CO_2 rose rapidly from 2·02 to 12·76 per cent. while the internal concentration of O_2 fell from 17·47 per cent. to 0·66 per cent. on the eighth day, with a recovery to 2·49 per cent. on the ninth day when the experiment ended. On examination the pulp was found to be soft and rotted by *Botryodiplodia theobromae*, a wound parasite whose pathogenicity to the banana, even at 53° F., is well known.

VI. Observations on Transpiration

The trend of relative transpiration rate (estimated as total loss in weight) of bananas at 85° F. has been illustrated in an earlier paper in this series (Leonard, 1940). Similar data have been collected for bananas at the lower temperatures considered in the present contribution and are illustrated in Figs. 1, 2 and 22–6.

In general, the transpiration trends are considerably less well marked. Fig. 1 shows the rates of respiration and transpiration of a '¾-full' finger maintained at 53° F. for 15 days and subsequently at 68° F., the atmosphere of the respiration chamber being saturated throughout. The transpiration rate shows an initial rapid fall followed by a steady level with a rise to a higher level on transfer to 68° F., a further rapid rise at the climacteric and a final slow rise in late senescence when the skin showed fungal attack. The relation-

ship in time between changes in respiration and transpiration rate noted in the earlier paper is thus maintained at these lower temperatures. Fig. 22 shows the transpiration rates for two corresponding fingers exposed to the room
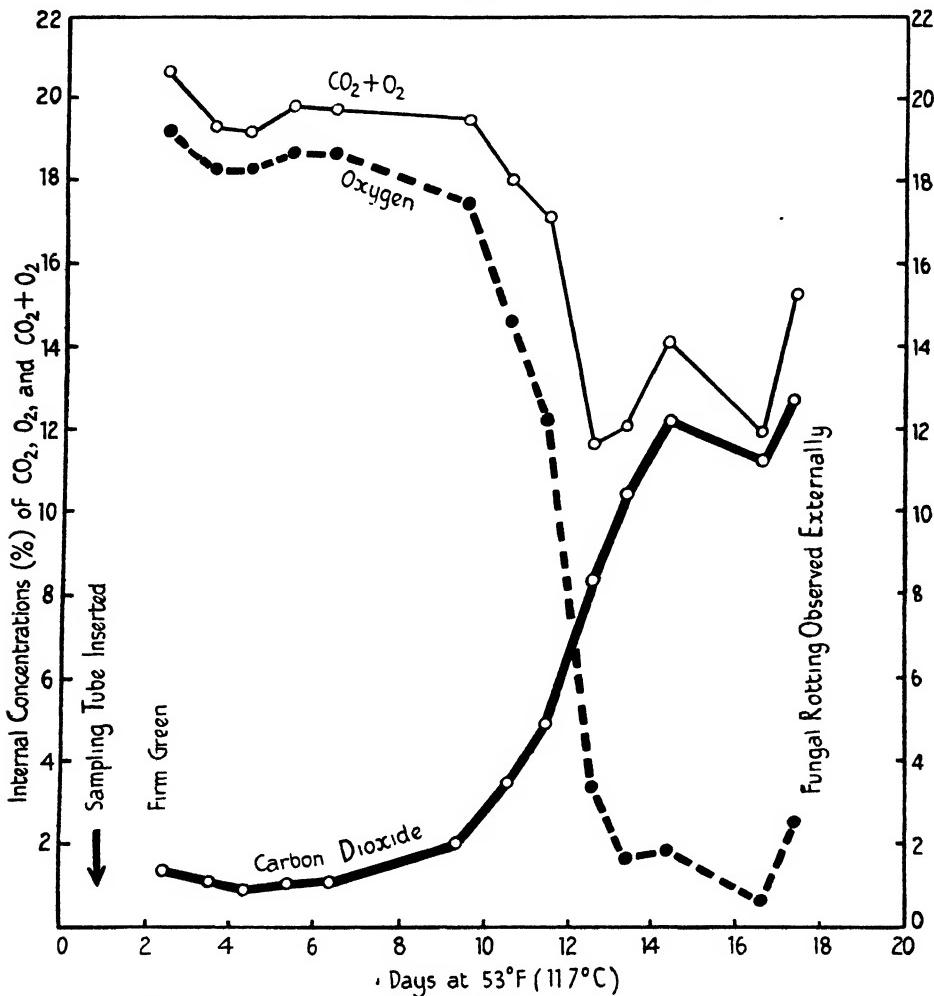
FIG. 21. Percentage internal concentrations of CO_2, O_2, and (CO_2+O_2) for a 'heavy ¾-full' banana at 53° F. (approx.) and 85 per cent. R.H. which developed fungal rotting in the sampling tube bore, for comparison with normal curves.

humidity (approx. 85 per cent. R.H. at both temperatures), together with the temperature and humidity records during the period at 68° F. The records for the period at 53° F. are given in Fig. 23 (1 to 15 days). Figs. 22 and 23 give the environmental conditions for the fruit of which the internal gas concentrations are shown in Figs. 3, 4, 5, 6, 7 and 8. The very considerably higher transpiration rate at the lower humidity is evident, and also the transition effect on transfer to 68° F., the rise in rate at the climacteric, and the
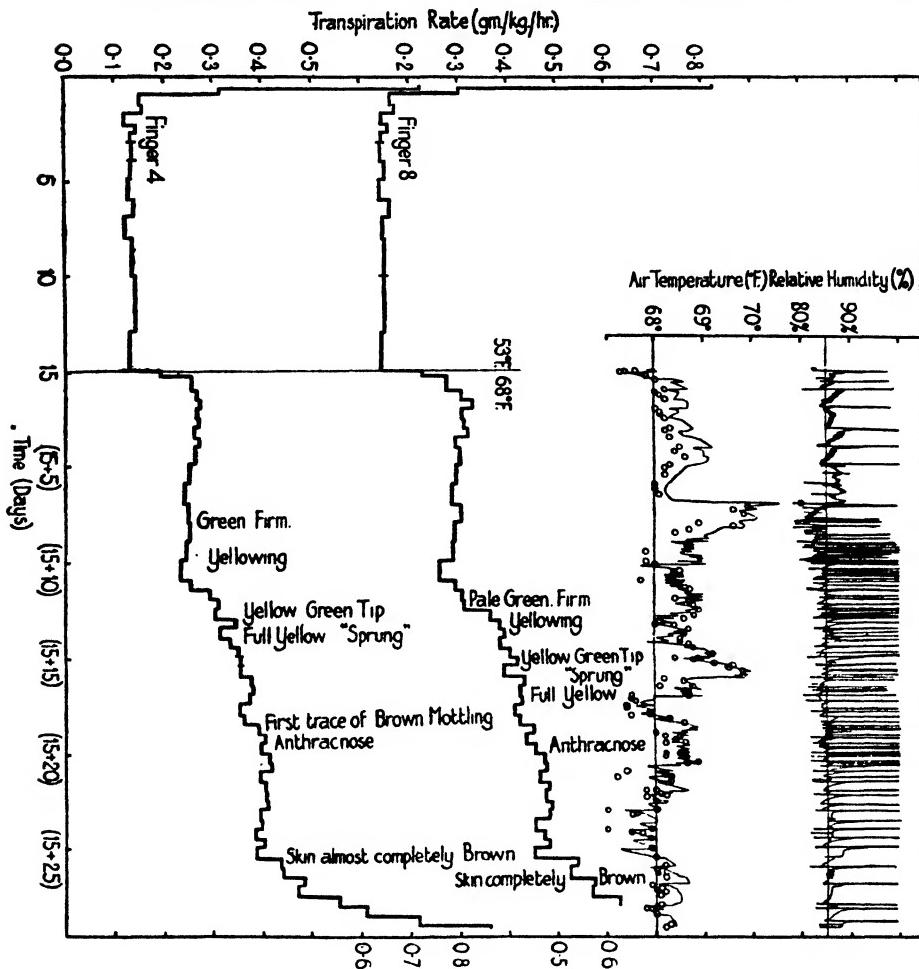
FIG. 22. Transpiration rates of two '¾-full' fingers at 53° F. (approx.) and 85 per cent. R.H. (approx.) for 15 days, with subsequent ripening at 68° F. (approx.) and 85 per cent. R.H. (approx.). For temperature and humidity records at 53° F. see Fig. 23; temperature and humidity records at 68° F. are indicated.

final rapid rise during late senescence. The following additional data are given:

TABLE VI

	R.H. 100% (Fig. 1).		R.H. 85% (Fig. 5).	
	Finger 7.	Finger 3.	Finger 4.	Finger 8.
Initial weight (gm.) . .	132·00	135·08	131·71	126·97
Final weight (gm.) . .	119·25	121·25	97·87	92·48
Loss (% of initial weight) .	9·66	10·24	25·69	27·16
Final Pulp/Skin weight ratio	2·24	2·50	3·63	3·10
Transpiration rate (gm./kg./hr.): 53° F. .	0·035	0·04	0·135	0·150
68° F. { Preclimacteric .	0·075	0·085	0·275–0·250	0·310–0·290
{ Postclimacteric .	0·140	0·160	0·355–0·410	0·420–0·485
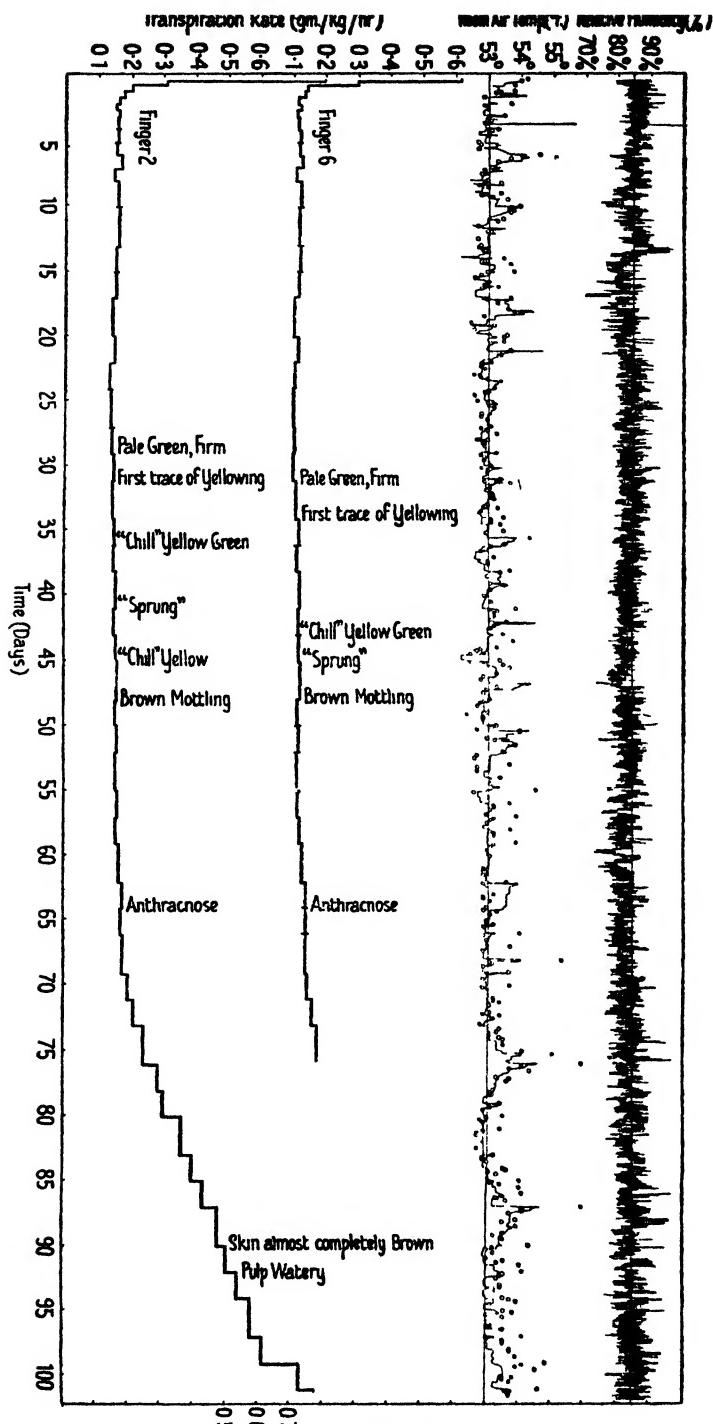
FIG. 23. Transpiration rates of two '¾-full' fingers at 53° F. (approx.) and 85 per cent. R.H. (approx.) continuously. Temperature and humidity records are indicated.

At 53° F. and 100 per cent. R.H. continuously, the transpiration rate (Fig. 2) showed no marked rise with the respiration climacteric but a very slight, slow and sustained rise throughout the storage period. The corresponding transpiration rates for two fingers exposed at 85 per cent. R.H. are given in Fig. 23 together with the temperature and humidity records of the room atmosphere: these give the environmental data for the fruit of Figs. 9, 10, and 11. The following additional data are appended:

TABLE VII

	Finger 2.	Finger 6.
Initial weight (gm.)	135·16	134·23
Final „ „	74·72	106·60
Loss (% of initial weight)	44·70	20·59
	(101·5 days)	(76·5 days)
Pulp/Skin weight ratio	3·15	3·41
Transpiration rate (gm./kg./hr.):		
Preclimacteric	0·15	0·12
Postclimacteric	0·16	0·14

It is seen that no marked rise occurs at this temperature and humidity until, with increasing browning and anthracnose development in the skin, a final senescent rise occurs.

Very similar trends of transpiration rate were found for individual hands of '¾-full' bananas at 53° F. and 68° F. or 53° F. throughout, with 85 per cent. R.H.

Data on the transpiration rates of 'heavy ¾-full' fruit are also available. Fig. 24 shows transpiration rates for two fingers from the same hand and row as those of Figs. 12 and 13 (section IV (*b*)) at 53° F. for 11 days and then at 65° F., with relative humidity 85 per cent. throughout. The environmental records at 53° F. are those for days 51·5 to 62·5 of Fig. 23. The trend is seen to be similar to that in Fig. 22. Additional data are given in Table VIII.

TABLE VIII

	Finger B.	Finger D.	Finger F.
Initial weight (gm.)	213·70	205·54	224·05
„ Pulp/Skin weight ratio . . .	—	—	1·90
Final weight (gm.)	143·06	156·44	—
Loss (% of initial weight) (11+29·5 days) .	33·06	23·89	—
Pulp/Skin weight ratio	2·50	4·36	—
Transpiration rate (gm./kg./hr.):			
53° F.	0·14	0·15	—
68° F. {Preclimacteric	0·23	0·23	—
{Postclimacteric	0·28	0·28	—

Similar trends of transpiration rate at 53° F. for 11 days and then at 65° F. were found for individual hands of 'heavy ¾-full' bananas. 'Heavy ¾-full' fingers and hands at 53° F. continuously give records similar to those of Fig. 23.
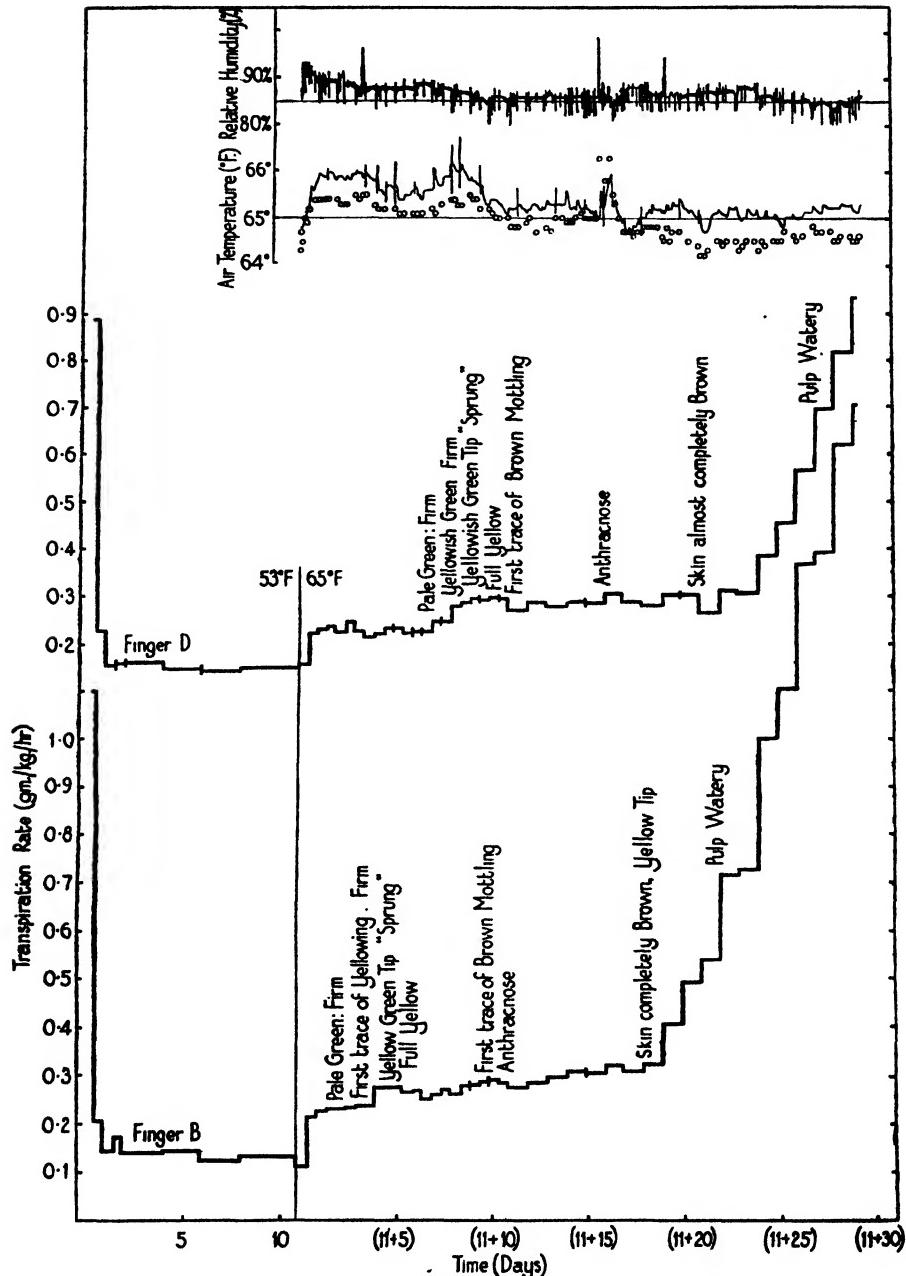
FIG. 24. Transpiration rates of two 'heavy ¾-full' fingers at 53° F. (approx.) and 85 per cent. R.H. (approx.) for 11 days with subsequent ripening at 65° F. (approx.) and 85 per cent. R.H. (approx.). For temperature and humidity records at 53° F. see Fig. 23 (days 51·5 to 62·5); temperature and humidity records at 65° F. are indicated.
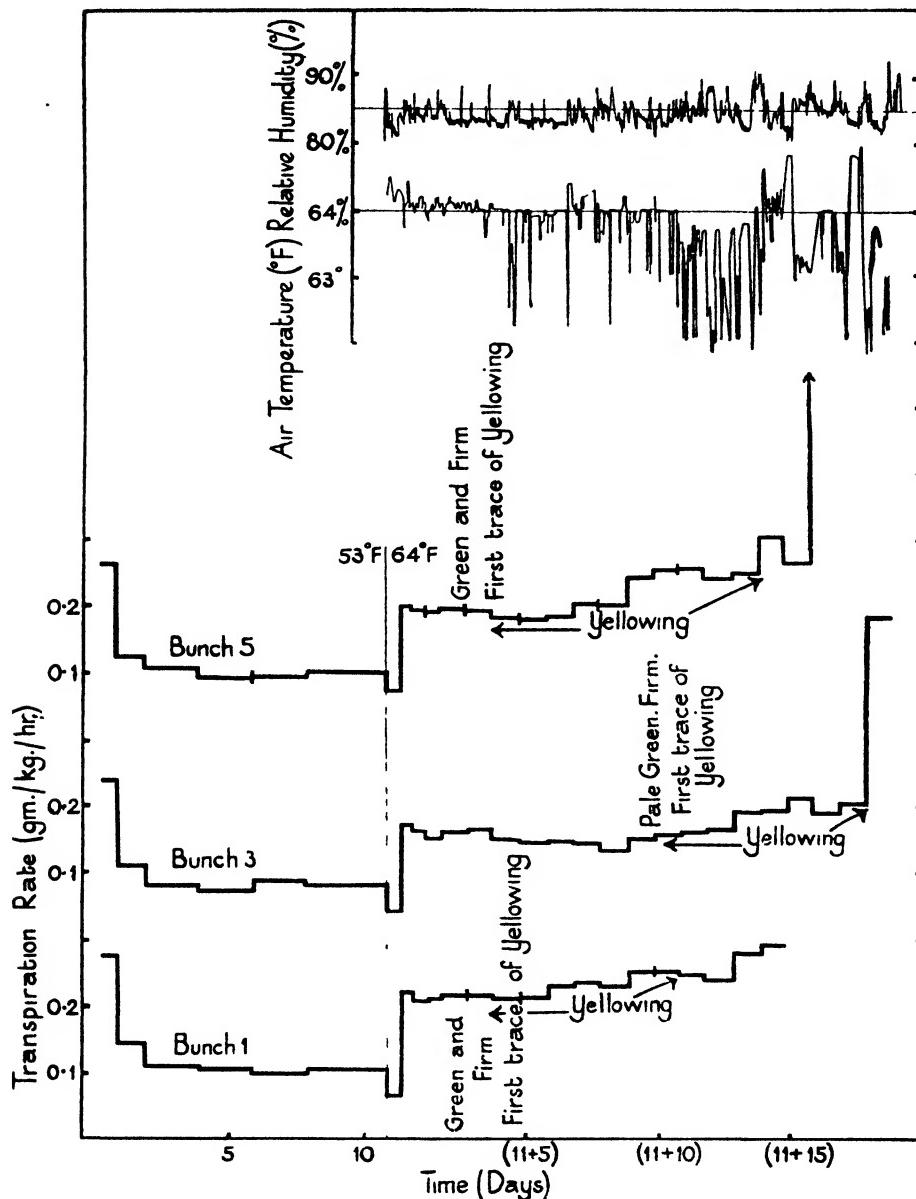
FIG. 25. Transpiration rates of three bunches of 'heavy ¾-full' bananas at 53° F. (approx.) and 80 per cent. R.H. (approx.) for 11 days with subsequent ripening at 64° F. (approx.) and 85 per cent. R.H. (approx.). For temperature and humidity records at 53° F. see Fig. 26; temperature and humidity records at 64° F. are indicated.

For three bunches Fig. 25 gives the transpiration rates and temperature and humidity records at 64° F. (another storage room). The records for the first 11 days at 53° F. are given in Fig. 26. As at 85° F. (Leonard, 1940), the climac-

teric at 64° F. is marked by a progressive rise in rate during the acropetal spread of ripening: the transpiration trend during the final phase of senescence cannot be obtained owing to fingers dropping from the over-ripe proximal hands. Additional data are given in Table IX.

TABLE IX

	Bunch 1.	Bunch 3.	Bunch 5.
Initial weight (kg.) . . .	26·59	34·42	33·63
Final „ „ . . .	23·71	30·89	29·01
	(11+15 days)	(11+19 days)	(11+16 days)
Loss (% of initial weight) . .	10·81	10·28	13·74
No. of hands	9 .	11	10
Transpiration rate (gm./kg./hr.):			
53° F.	0·11	0·08	0·10
64° F. (Preclimacteric) . .	0·21	0·15	0·19

Fig. 26 gives corresponding data for bunches at 53°–56° F. throughout. A slow rise in transpiration rate is seen but this may be in part due to the slow rise in mean temperature. Additional data are as follows:

TABLE X

	Bunch 2.	Bunch 4.	Bunch 6.
Initial weight (kg.) . . .	31·81	30·35	25·76
Final „ „ . . .	26·65	25·24	22·10
	(53 days)	(55 days)	(50 days)
Loss (% of initial weight) . .	16·22	16·85	14·22
No. of hands	10	11	9
Transpiration rate (gm./kg./hr.):			
53°–54° F. (Preclimacteric). .	0·10	0·11	0·11
55°–56° F. (Postclimacteric) .	0·15	0·13	0·13

In general the changes in transpiration rate during the climacteric are considerably less marked at the low temperatures employed in these experiments than at tropical temperatures. Nevertheless, the same general type of trend is found at 68° F. or 65° F., i.e. a rise from a low to a higher level of transpiration, but the transition effect, rapid rise and slight fall, is considerably suppressed. At 53° F. only a very slow trend of increasing transpiration rate is observed until the final senescence and browning of the skin. The time relationship with respiration rate changes (for fingers) is the same as at tropical temperatures.

Smith (1933) has given for bananas at 54·5° F. (12·5° C.) a curve of rate of loss in weight against time showing a slowly declining rate at 90 per cent. R.H. and a slightly rising rate at 100 per cent. R.H. over a period of 20 days. Judged by the initial weight, the fruit was considerably less than '¾-full'.

VII. DISCUSSION

It has been pointed out that the ripening of a detached fruit must differ in certain essential respects from the ripening of a fruit still on the tree (Wardlaw and Leonard, 1940). For the apple, Kidd and West (1930) found that the phenomenon of the rise in respiratory activity does not in all cases set in
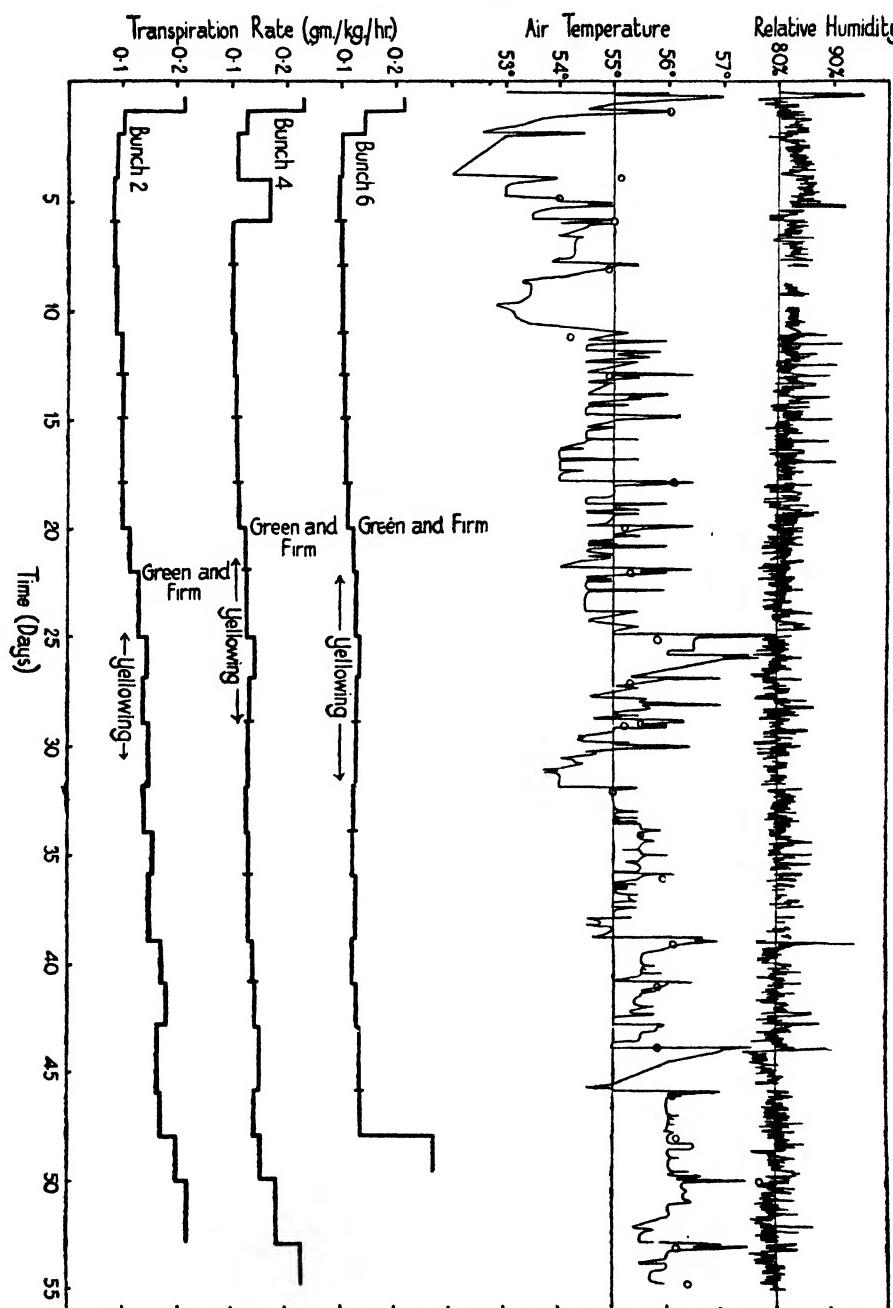
FIG. 26. Transpiration rates of three bunches of 'heavy ¾-full' bananas at 54° F. (approx.) and 80 per cent. R.H. (approx.) continuously. Temperature and humidity records are indicated.

immediately after gathering. They suggest that it is, in fact, 'a phenomenon which is not associated causally with separation from the tree. It may be initiated some considerable time after separation or it may occur on the tree before gathering. It appears thus to be inherent in the senescent drift of metabolism in the apple.' In the pear (Kidd *et al.*, 1940) the upward drift of change in starch and sorbitol is immediately reversed on gathering. At tropical temperatures it has been shown that the severing of a banana from the plant is attended by important changes in the respiration and transpiration rates, and that biochemical changes accompany these. The obvious differences between the different fruits need not be stressed.

The expeditious placing of freshly harvested fruit in cold storage is the normal current commercial procedure. It has been shown above to have a marked effect on retarding ripening changes throughout the subsequent storage period, and also to minimize the changes taking place during the immediate post-harvesting period.

Kidd and West found for apples (1925) and pears (1936, 1937) that temperature has little effect on the duration of the preclimacteric period in storage but a considerable effect upon the duration of the climacteric rise; the time taken by apples in passing from the minimum to the maximum of the climacteric rise in respiratory activity is unexpectedly long at low temperatures (Kidd and West, 1924). They also suggest (1934) that the slow continuous rise in respiration rate of apples at 34° F. represents a delayed climacteric, homologous with the much more rapid phenomenon observed at higher temperatures. They observed the same relationship between the change in ground colour from apple-green to yellowish-green and the rise in respiratory activity in spite of the marked extension in time. In the banana, lower temperatures delay the onset of the climacteric rise, especially in the higher regions (tropical temperatures down to 68° F.): the effect is less marked below 68° F. The duration of the rise is, however, considerably extended at 53° F. as compared with 68° F. or 65° F.

The readjustments in the internal concentrations of CO_2 and O_2 which take place on transferring fruit from tropical temperatures to 53° F. (11·7° C.) have been demonstrated. They involve a decrease in the concentration of CO_2 and an increase in that of oxygen which is not *pro rata*, oxygen concentrations of approximately 18 to 20 per cent. having been recorded at the lower temperature. If the temperature coefficients of the several metabolites whose activities normally involve the utilization of oxygen are not identical, it follows that those, the activities of which are only slightly affected at the lower temperature, may proceed at relatively higher rates because of the presence of the higher partial pressure of oxygen. If this hypothesis can be confirmed it will afford an explanation of certain abnormal ripening trends, e.g. chilling, observed in fruit whose cold storage period has been prolonged beyond the normal commercial practice or, as in the present experiments, where ripening has been carried on at 53° F. throughout.

The differentiating effect of temperature on metabolic processes has been discussed by Kidd and West (1937) who comment that while broadly the same developmental stages are passed through at all temperatures, there cannot be an exact correspondence between the chemical and physical state of fruits which are in the same stage but which have different histories as regards temperature. In a previous discussion of the same effect they had stated (1924): 'With the same change in temperature the rate of one reaction is doubled, that of another trebled. It is clear, therefore, on purely theoretical grounds that in a complex of interdependent reactions, such as is represented by the living organism, the effect of a change in temperature should be dual, first upon the net speed and secondly upon the balance and constitution of the complex.'

With reference to the effect of a lower temperature on the internal concentration and tissue content of CO_2 it has been shown that whereas the change from 85° F. to 53° F. reduces the internal gaseous concentration by approximately 100 per cent., the CO_2 content of both pulp and skin remains unaltered. Only a partial analysis of this situation is possible at the present stage, but it may be noted that whereas the rate of production of CO_2 in metabolism is diminished at the lower temperature the capacity of tissues for retaining the gas is increased. This is to be expected in a purely physical system but obviously in the biological system many other factors such as changes of permeability may be involved.

The importance of associated gas phenomena on the initiation of ripening has been considered at some length in a previous paper, where it is noted that the onset of the climacteric is denoted by a sharp downward trend in the curve of internal oxygen concentration from an earlier steady or slightly downward trend. Whether for different grades of fruit ripening at different temperatures there are threshold values of internal oxygen concentration which are of critical importance in the initiation of ripening changes cannot yet be definitely stated, but it will be noted that the several records presented in this paper would appear to lend support to the general hypothesis suggested (section III (*c*), Fig. 9, and I (*b*), Fig. 18).

Kidd and West (1932) found that an increase in the oxygen concentration of the atmosphere surrounding bananas of 30–50 per cent. above that of normal air accelerated their ripening, whereas reduction of the concentration to 10 per cent. or less produced a delay, these effects being similar to those with apples.

In general the several phases of ripening whether at 53° F., 65° F., or 68° F., as considered here, correspond with those already observed in the 'normal' ripening of heavy grades of fruit at tropical temperatures, i.e. ripening change is initiated in the central placental region and proceeds outwards through the pulp to the skin. Certain differences, however, call for comment. Before dealing with these in detail it should be noted that although the disappearance of starch at the lower temperatures is greatly retarded, nevertheless, fruits held until final senescence at 53° F. eventually show an almost complete disappearance.

In a fruit ripening at tropical temperatures a definite sequence in time has been consistently found to exist in the attainment of certain well-defined stages in the progress of ripening change, notably that at the climacteric (i) the attainment of minimal O_2 concentration precedes (ii) the attainment of maximal CO_2 concentration (and of the new carbon dioxide content) which in turn coincides with (iii) the first evidence of skin coloration and which slightly precedes (iv) the attainment of the 'sprung' condition. In fruit ripening at lower temperatures some displacement of these stages has been found: at 65° F. or 68° F. after storage at 53° F. a much closer coincidence of the four points mentioned above is found, while in fruit ripened throughout at 53° F. the climacteric level of internal concentration of carbon dioxide (ii) and the onset of skin coloration (iii) are attained considerably in advance of (i) the minimal oxygen concentration, while the 'sprung' condition (iv) follows almost immediately on the latter. Further, whereas in fruit at tropical temperatures the condition recognized as 'eating-ripe' follows almost immediately on the 'sprung' condition, this is considerably delayed at 65° F. and 68° F. and very greatly retarded at 53° F.

The climacteric at tropical temperatures is denoted by well-defined peaks in the curves of both respiration rate and internal concentration of CO_2. It has been indicated (Wardlaw and Leonard, 1940) that the essential feature of the climacteric is that it represents a change from a lower to a higher rate of metabolism, the spectacular rise to and fall from the peaks being to some extent in the nature of physical transition effects. At lower temperatures, 65° and 68° F., such physical transition effects are less evident, while at 53° F. they are practically absent. An exposition of some of the factors possibly involved has already been given. Recently Boswell and Whiting (1940) have suggested that in any change in the external conditions which results in a change in the rate of production of carbon dioxide account must be taken of changes which occur in the large quantity of carbon dioxide bound in the tissue (of potato tubers) and on the basicity of the tissue through change in the nature of the end products.

Smith (1933 *a*) comments that measurements of the rate of generation of heat of carbon dioxide by bananas made after their arrival in the United Kingdom must be treated with reserve as indicating the conditions met with on board ship, but so far as they go they indicate a rate of production of heat at the time of arrival of the order of 150 B.Th.U./ton/hr. (15 mgm./kg./hr.); within a few days, if the bananas continue to be stored at a temperature of 53°–54° F., this figure rises to a maximum value of the order of 450 B.Th.U./ton/hr. (45 mgm./kg./hr.) and thereafter declines again.

The general symptoms of chilling in bananas have been fully described elsewhere; it has also been shown that chilling is not the result of rapid cooling to a specified low temperature but is a cumulative effect of the duration of exposure at that temperature (Wardlaw and McGuire, 1931). In the prolonged experiments described in this paper when fruits were maintained at

53° F. for periods up to 105 days, observations on chilling have been amplified. The following points may be noted: (i) the development of chilling symptoms closely accompanies the progress of ripening; (ii) an additional internal symptom is the appearance of a black discoloration in the placental region; (iii) skin coloration passes from a ruddy yellow discoloration to a deep bronze colour until the necrotic brown colour of final senescence supervenes; (iv) the failure to ripen which has been attributed to chilled fruit is a relative term with obvious commercial uses, but in the light of these observations is seen to be inexact in that a complete though not necessarily 'normal' ripening involving the almost complete disappearance of starch eventually takes place. Although data on changes in respiration rate and carbohydrate metabolites in chilled fruit are now available the evidence indicates that further work along special lines will be necessary before a critical account of the initiation and progress of chilling injury can be given.

VIII. Summary

1. The respiration of individual banana fruits of standard commercial grades during storage at 53° F. (11·7° C.) and ripening at controlled temperatures (65° F. and 68° F.) was investigated by methods yielding data on respiration rates, on internal concentrations of CO_2 and O_2, and on the CO_2 content of tissues.

2. During storage at 53° F. and 100 per cent. R.H. for periods normal for the grades of fruit, respiration proceeded at a steady rate after an initial fall. On transference to 65° F. (18·3° C.) or to 68° F. (20° C.) there was a well-marked transition effect; this was followed by a steady rate until the onset of the climacteric, when the rate rose to a peak value, descended to a new level, again rose slightly and finally fell off steadily during senescence.

3. By contrast with the above, similar fruits at 53° F. and 100 per cent. R.H. throughout maintained a steady respiration rate until the onset of the climacteric, when the rate increased to 2·5 times its previous value in the course of seven days; thereafter the respiration rate showed a slow decline, rising slowly again during final senescence. Such fruits showed a marked development of 'chilling' injury but ripening, though much protracted, was eventually completed.

4. The internal concentrations of CO_2 and O_2 in the same or similar fruits were also observed under the storage conditions indicated, with the following results: on transferring fruits from 85° F. (29·4° C.) to 53° F. there was a decrease in the internal concentration of CO_2 and a considerable increase in that in O_2. Approximately steady levels were then maintained during storage at 53° F., but on transference to 68° F. or 65° F. transition effects were observed, followed by the establishment of new levels, i.e. higher CO_2 and lower O_2 concentration. With the onset of ripening the internal concentrations of CO_2 and O_2 rose and fell respectively, the peak of internal CO_2 concen-

tration approximating closely in time with the peak of respiration rate; during the climacteric phase there was a recovery in the O_2 concentration from minimal values, and a decline in the CO_2 concentration from the peak value. The increased resistance which tissues offer to the movement of gases as a result of ripening changes was attended by a decline in the O_2 concentration and an increase in the CO_2 concentration during sensecence. These data and observations on tissue changes have been compared with those occurring during ripening at tropical temperatures; the part which O_2 may play in the initiation of ripening changes is discussed.

5. Relative to the striking changes taking place at the climacteric, the pre-climacteric trends in internal gas concentrations at 53° F. and 68° F., or 65 °F., show only small changes; nevertheless, these are consistently in the direction of increasing concentration of CO_2, indicating a changing rate of metabolism; this is confirmed by estimations of the carbohydrate metabolites.

6. The internal gas concentrations observed in fruits held at 53° F. throughout were very different from those described above. The onset of ripening was denoted by a slow rise in the CO_2 concentration to a comparatively low maximal value, this being in the nature of a sustained level rather than of a climacteric peak. During this period the internal O_2 concentration, as at higher temperatures, slowly descended to a minimal value with the exception that such minimal value was attained *after* the peak in the internal CO_2 curve and was immediately followed by the attainment of the 'sprung' condition. During the subsequent period at 53° F. when the bronzed 'chilling' colour became very conspicuous the fruit slowly ripened, the curve of internal CO_2 concentration falling then rising and that of O_2 rising then falling as final senescence was approached.

7. At all temperatures, the curve for the CO_2 content of fruits during storage and ripening was characterized by steady values during the unripe stage and by a peak value coinciding with the peak value of internal concentration of CO_2; thereafter the curve of CO_2 content declined but subsequently rose during senescence.

8. Observations on transpiration during storage at 53° F. and ripening at controlled temperatures are considered in some detail. In general the trans-piration trends are considerably less well marked at lower temperatures than those previously obtained at tropical temperatures. Nevertheless, a rapid rise in transpiration rate at the climacteric, at 68° F., was again demonstrated. The relationship in time between changes in respiration and transpiration rates noted in previous studies was again demonstrated at the lower temperatures employed.

9. The results of these studies are discussed in relation to the findings of other investigators for other fruits, e.g. apples and pears. Consideration is also given to the significance of the readjustments of the internal concentrations of CO_2 and O_2 which take place on transferring fruit from tropical temperatures to 53° F. (11·7° C.), and their relation to the development of abnormal ripening trends, e.g. in 'chilled' fruit. Other relevant topics are also discussed.

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A Sixth Contribution to our Knowledge of the Anatomy of the Cone and Fertile Stem of Equisetum

BY

ISABEL M. P. BROWNE

With five Figures in the Text

I. Introduction

IN 1912 Professor J. H. Schaffner (1912, p. 21) described a new species of Equisetum, *E. kansanum*, which had previously been regarded as a form of *E. laevigatum* A. Br. characterized by annual aerial stems. He pointed out that in the new species not only were the aerial stems annual and smoother than in *E. laevigatum*, but that the cones instead of ending, as did those of the latter species, in a rigid apical point had obtuse or merely acute apices.

A short account of the anatomy of the aerial stem has recently been published (Browne, 1939).

The present paper embodies the results of a study of the cone of *E. kansanum* and of the fertile stem at the level of insertion of the annulus. Abundant material, duly fixed and preserved, was sent to me from Ohio by Professor Schaffner, whom I wish to thank most sincerely for his kindness.

As neither the original specific diagnosis nor, so far as I am aware, any subsequent publication speaks of the dimensions of the cones of *E. kansanum* it may be well to state that the more or less mature cones sectioned by me varied from about 11 mm. to about 20 mm. in length, measured from the insertion of the annulus; and that at their widest their diameter, inclusive of the short sporangiophores, was from $5\frac{1}{2}$ to nearly $6\frac{3}{4}$ mm.

Eight complete cones were cut up into sections 14 μ thick. These cones will henceforward be alluded to as cones A, B, C, D, E, F, G, and H. Cones A and C were cut longitudinally, all the others transversely. Cone E was abnormal in that it was branched. Its asymmetry rendered it impracticable to make a satisfactory two-dimensional reconstruction of its vascular system, but such reconstructions were made of the complete steles of the five cones B, D, F, G, and H.

II. General Description of the Cones

1. *The endodermis.*

In the axis of the cone the vascular bundles are surrounded by an endo-

dermal sheath of relatively large cells, the walls of which are somewhat thickened, especially on their outer sides where they are in contact with the cells of the outer ground-tissue. The cells of the sheath do not show typical endodermal thickenings on their radial walls, but they are easily distinguishable by their size and more rigid lignified walls. The distribution of the cells composing the sheath is continually changing, since not only may its cells be locally duplicated (in which case the outer ones are usually markedly larger) but, when the bundles anastomose, the cells of the sheath between them are replaced by metaxylem. When such a vascular band breaks up again into separate bundles new sheath-cells develop between these. And as the bundles become more widely separated by parenchyma the sheaths follow their contours. The sheaths have, therefore, clearly no morphological value. There is in the cone no common endodermis separating cortex from stele; the central ground-tissue passes gradually into the cortex.

2. *Structure of the vascular bundles.*

In the axis of the cone of *E. kansanum* protoxylem and metaxylem are rather more often than not in contact with one another. The metaxylem elements tend to form a band lying towards the periphery of the bundle, usually one, sometimes two, more rarely three cells deep. This band may be in parts interrupted by one or more parenchymatous elements. Adjacent and internal to it there is usually a group—sometimes more than one group—of metaxylem-tracheides; these generally reach inwards to the protoxylem or to the edge of the carinal canal that may replace the latter. It is, however, not at all uncommon for protoxylem and metaxylem to remain separated by a few parenchymatous elements; and there is much variety in the distribution of the metaxylem in the bundle. Occasionally one, more rarely two, much larger tracheides are developed internally to the protoxylem and these must presumably be regarded as centripetal xylem. Even where protoxylem and metaxylem are in contact, the tracheides of the former are definitely smaller and less strongly lignified than those of the latter. In sections from the lower part of the cone the protoxylem of some of the bundles is often replaced for a certain distance by a small carinal canal, while in others it is, at the same level, persistent. Such differences may even be observed between bundles temporarily united by an increase of their metaxylem. The way in which in the same protoxylem-strand the tracheides may be for a time replaced by a small canal, then reappear again and give way to a smaller or larger canal, in its turn superseded by a group of persistent tracheides, and the way in which the protoxylem has been destroyed in some bundles and is persistent, at the same level, in others—these facts point to the conclusion that in the cone of *E. kansanum* the protoxylem develops later in some strands than in others, and that even in the same strand its differentiation does not always proceed regularly.

3. *The central ground-tissue.*

Except near the apex the centre of the axis of a mature cone of *E. kansanum* is usually occupied by a cavity varying in extent according to the age and width of the cone. In the branching cone, cone E, there was no definite cavity, the centrally situated cells being merely somewhat torn. In cones A, D, and H a persistent central core consisting of very small sclerized cells surrounded by a few small parenchymatous cells was found for a short distance. In cone A it was situated between the fourth and fifth whorls and its vertical extent was about 2·5 mm.; while in cones D and H it was near the level of the lowest whorl of the cone and was from 1·3 to 1·5 mm. long. In cone D there was a certain amount of apparently mucilaginous matter above and below this sclerized tissue. The core and the small parenchymatous cells around it are connected with the two to six—locally more—layers of parenchyma that persist internally to the bundles by means of slender radial bands, usually two cells wide, the individual cells of which have become much elongated radially, while most of the other cells of the ground-tissue have perished. In cone C, though no definite central core was present, an unusual amount of parenchyma persisted at the centre of the axis between the levels of the third and fourth whorls.

In the cone of *E. kansanum* the ground-tissue outside the ring of bundles is remarkably narrow radially, usually about 0·25 mm., occasionally about 0·33 mm. wide.

III. THE SPORANGIOPHORES AND THEIR TRACES

The stalks of the sporangiophores of *E. kansanum* are rather short. The number of tracheides in a cross-section of a trace varies much even in adjacent traces of the same whorl. Often there are from 10 to 15, though there may be more or occasionally fewer. In transverse section the vascular elements are seen to be surrounded by a sheath, often locally double, of large cells. The outermost metaxylem-elements of the trace are from four to five times as wide as the protoxylem-elements. The latter are often in part replaced by a very small canal; a few parenchymatous cells of much the same size as the protoxylem-tracheides may be found among these.

The traces of the sporangiophores of nine species of Equisetum have been re-examined in order to compare them with those of *E. kansanum*. Among the species studied only *E. giganteum* L. and *E. debile* Roxb. have sporangiophore-traces that contain more numerous tracheides than do those of *E. kansanum*. In *E. giganteum* the traces whilst passing through the cortex show in cross-section from 17 to 22 tracheides, of which five or six are protoxylem. In *E. debile* the traces had in cross-section from 13 to 26 tracheides, most of them of the nature of metaxylem. At the other end of the scale are found the traces of
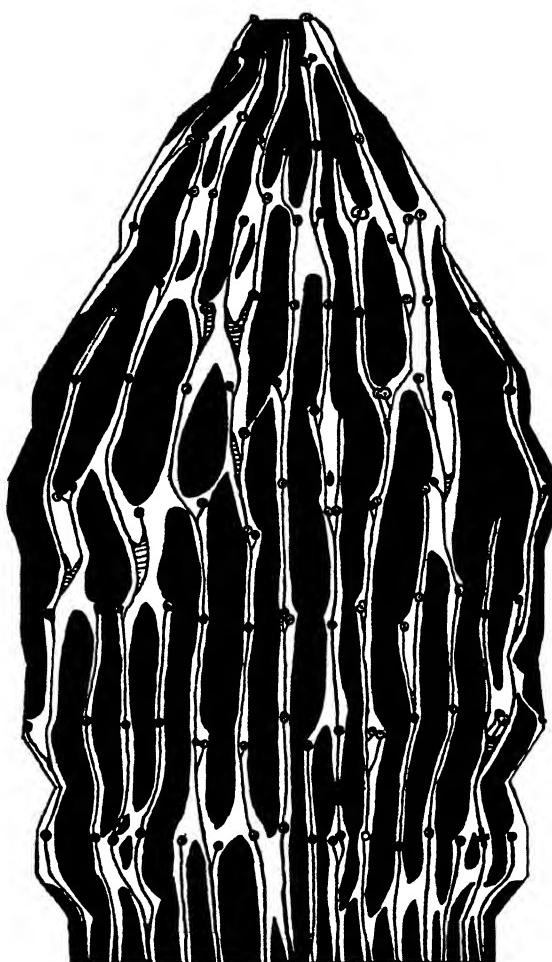
FIG. 1. Reconstruction of the stele of cone B of *Equisetum kansanum* Schaffner, × 12. In this and the following reconstructions of the steles of cones the parenchyma is black; the protoxylem is shown by a line and the presence of a small canal left by its destruction by transverse lines on white. The metaxylem is left white. Normal traces are shown by small circles with a central dot for the protoxylem. Traces in which the metaxylem joins up with that of the axis, but in which the protoxylem dies out without penetrating at all into the stele, are shown by a circle with a line across it; and those in which both protoxylem and metaxylem penetrate into the stele, but in which the former fails to connect with the protoxylem of the axial bundle, are represented by a circle without a line or a dot (traces of the last two types are not present in the cone shown in this figure). Incoming traces dying out in the cortex are shown by a cross, black on white and white on black, on the part of the stele opposite to which they die out.
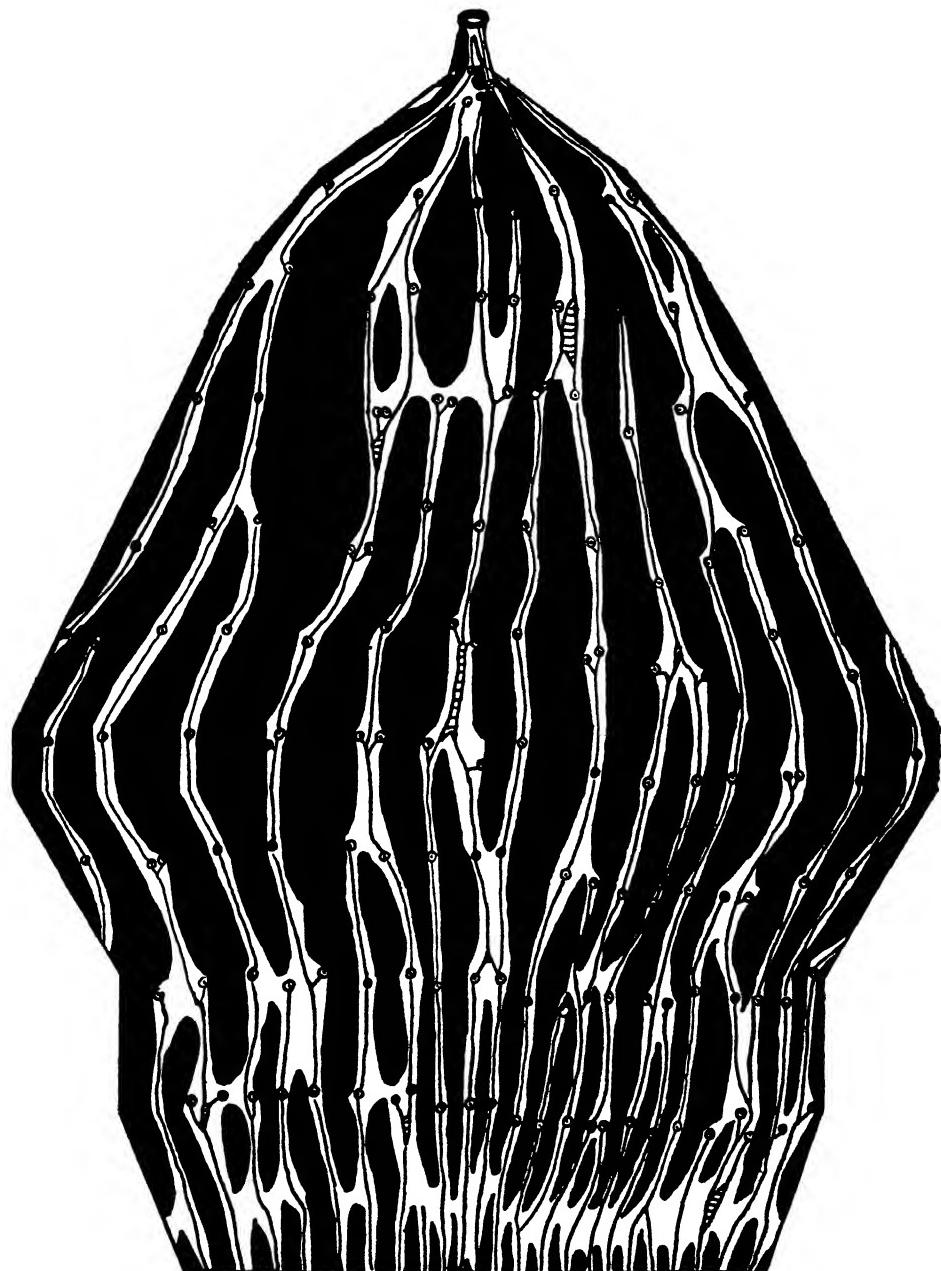
FIG. 2. Reconstruction of the stele of cone D of *E. kansanum*, ×12.
For explanations, see Fig. 1, p. 428.

E. maximum Lam., of *E. arvense* L., of *E. silvaticum* L., and of *E. palustre* L.
In these species the sporangiophore-trace is as a rule small, its xylem con-
sisting exclusively or almost exclusively of protoxylem.[1] In my specimens of
E. limosum the amount of xylem in the traces of the sporangiophores was
small; the tracheides were nearly all of the nature of protoxylem, though
occasionally there was a small admixture of metaxylem. In the traces
of the sporangiophores of *E. hyemale* L. the contribution of the meta-
xylem is relatively greater. In *E. variegatum* Schleicher the protoxylem
passes insensibly into the metaxylem; but as in it some of the larger, more
peripherally, as well as some of the smaller, more centrally situated
tracheides pass out from the axis into the sporangiophores the traces of the
latter presumably contain elements corresponding to both proto- and
metaxylem.

On general anatomical grounds it seems clear that the more primitive kind
of trace is that carrying off axial metaxylem as well as protoxylem.

Unusually small traces and small apparently stunted sporangiophores
are occasionally found in *E. kansanum*. The traces of the third sporangiophore
of the seventh whorl of cone H (which sporangiophore is concrescent with the
fourth) and of the eleventh sporangiophore of the third whorl of cone F are
very small, though the sporangiophores that they supply are of the normal
size. The fifteenth sporangiophore of the lowest whorl of cone B was re-
markably small; its vascular bundle did not penetrate into the axial stele (cf.
Figs. 1, 3, and 5). Though its trace is of the usual size, the seventh sporangio-
phore of the seventh whorl of this cone is smaller still and almost crowded out
by its neighbours. It bears on one side a sporangium about half the ordinary
size and containing normal-looking spores; and on the other side the rudi-
ment of an aborted sporangium, the cells of which stain very deeply. In con-
tact with them are found similar cells occupying the position of a sporangium,
but belonging to a neighbouring (the sixth) sporangiophore, the other
sporangia of which are normally developed. The tenth sporangiophore of the
fourth whorl of cone G was only about two-thirds of the length of its neigh-
bours and bore no sporangia. It seemed as though a part of the tissue that
normally develops into a sporangium had become concrescent with the stalk,
which was thickened. The xylem of this trace was very small in amount and,
in striking contrast to that of the normal traces of *E. kansanum*, consisted only
of protoxylem. This trace remained for a time adherent to the edge of the
stele and then pursued a steep upward and outward course into the
sporangiophore. In the head of the latter the vascular bundle divided and its
branches ran to the points where the sporangia would normally be (cf. Fig. 4,
p. 443).

Bifascicular sporangiophores are by no means rare in *E. kansanum*, at least
in my specimens. In some cases the bundles entering such a sporangiophore

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[1] For a highly exceptional case where this was so, also in *E. kansanum*, see p. 431.

have arisen by the precocious division of the trace in the cortex; but traces also often originate as two strands. These two strands may be given off from two separate bundles, or, where bundles have become temporarily united into a vascular band, from portions of the band belonging to different constituent bundles. The protoxylem-strands of such a bifascicular trace would naturally be given off from different axial strands of protoxylem. Much more often, so it would appear, the strands composing the xylem of a bifascicular trace arise from a single bundle, or from the part of a vascular band corresponding to a single vascular bundle. In such cases the axial strand of protoxylem gives off, a little below the departure of the trace, a branchlet of protoxylem which diverges slightly from the parent strand and, a little higher up, passes out as the protoxylem of one of the bundles of the trace; the second protoxylem of the trace may also be given off as a small branchlet arising a little below the level of departure of the trace, or it may pass out only at that level. In a bifascicular trace, or even in one which though monofascicular is given off deeply lobed and about to divide, the number of tracheides in the whole trace may be considerably more numerous than in ordinary traces.

The above remarks apply to sporangiophores which, whether monofascicular or bifascicular, are single in nature. But in *E. kansanum* concrescence of sporangiophores, either partial or complete, seems to be relatively common.[1] Such concrescence is always in a lateral direction; the resulting complexes may consist of two or occasionally of more members. A bifascicular sporangiophore, single in nature, may become concrescent with a monofascicular one or with another bifascicular one. In the case of such complexes the constituent sporangiophores generally receive their traces from different bundles—or, where there is a vascular band, from portions of the band corresponding to different bundles. Since, however, two entirely free neighbouring sporangiophores of a whorl occasionally receive their traces from one and the same bundle it is *a fortiori* probable that this may also occur when neighbouring sporangiophores have become concrescent.

As already noted, the degree of concrescence of the sporangiophores varies from partial fusion of the bases only of their stalks to an extensive union in which the fused heads come to form a complex, roughly oval in outline and as a rule more or less deeply, but sometimes only slightly lobed, the furrows indicating the surfaces of concrescence. It is not always easy to distinguish between a single, unusually large bifascicular sporangiophore and two very nearly completely concrescent monofascicular ones, especially as the head of a large single sporangiophore, whether bifascicular or not, may be somewhat lobed. As a rule the size of the member, its form and more especially the number of its sporangia enable one to distinguish between the two structures.

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[1] A considerably higher proportion of concrescent sporangiophores has been recorded in the lowest whorl of a cone of *E. maximum* (Browne, 1923); but in other whorls of this cone and in the cones of this species generally concrescence of sporangiophores does not seem to be as marked a feature as in *E. kansanum*.

Further indications may be afforded by the distance of the structure in question from the sporangiophores on either side of it and by the number of members in the whorl to which it belongs compared with the numbers in the whorls above and below, although the distribution of the sporangiophores, even when undisturbed by concrescence, is not sufficiently regular for either their position or their number to be *by itself* an exact criterion of their nature and number. But if all factors are taken into consideration it is only in a very few cases that a sporangiophoric structure may almost equally well be regarded as a single exceptionally large sporangiophore or as consisting of two concrescent members. In *E. kansanum* the commonest number of sporangia on a single sporangiophore seems to be five; but six is also a common, and four by no means a rare number. This last number is often found where one or both the neighbouring sporangiophores have six or seven sporangia and these press laterally on the sporangiophore in question. A complex of two members does not usually bear twice as many sporangia as a single sporangiophore, since no sporangia are borne on the surfaces of fusion. In cone A, however, one or two complexes formed by two nearly completely fused sporangiophores bore nine sporangia. Though two concrescent sporangia may bear but six sporangia, especially if crowded up by neighbouring sporangiophores, most sporangiophoric structures with this number of sporangia are clearly single in nature.

It might seem natural to look upon a high proportion of concrescent sporangiophores as a stage towards their diminution in number, a stage that might well have been reached if the diameter of the axis underwent proportionately greater reduction than the reduction in number of the foci for the development of sporangiophores, the latter thus becoming more closely approximated, which would facilitate their concrescence. In *E. kansanum* concrescence of sporangiophores manifests itself very unequally not only in different cones, but even in different whorls of the same cone; and this affords a means of testing the validity of the suggestion made in the last sentence. The following table gives particulars for each of the cones of which reconstructions of the stele have been made of the number of free sporangiophores, of those that are concrescent to a considerable extent, and of the number of bifascicular sporangiophores regarded as single in nature under their different whorls; it also gives the average extent of stelar circumference per sporangiophore for each whorl.[1]

[1] In this and the following tables the apical group of incompletely differentiated more or less concrescent sporangiophores has been left out of consideration. The amount of space available has been measured with reference to the circumference of the stele rather than with reference to that of the axis, because the sporangiophore-stalks are slightly dilated at their base and their tissues pass insensibly into those of the axis. This, combined with the slightly varying level of insertion of the sporangiophores, makes it almost impossible to obtain reliable measurements of the radial extent of the cortex. This is, however, small; and apart from the continual slight fluctuations mentioned does not seem to vary much, so that in taking the circumference of the stele as the basis for estimating the approximation of the sporangiophores to one another we would appear to have chosen a fairly reliable standard.

TABLE I

Cone.	Whorl.	Number of sporangio-phores in whorl.	Sporangio-phores mar-kedly con-crescent.	Bifascicular sporangio-phores single in nature.	Allowance (mm.) of Stelar circumference per sporangiophore.
B	1	16	0	1	0·329
	2	13	0	3*	0·405
	3	14	2	1	0·4
	4	11	2	5*	0·53
	5	12	0	1	0·44
	6	11	4	1	0·391
	7	10	4	0	0·35
	8	8	2	0	0·3
Total for B	8	95	14	12	0·384 (average)
D	1	25	0	0	0·25
	2	21	2	0	0·357
	3	18	2	1	0·5
	4	18	6	0	0·54
	5	15	0	1	0·6
	6	13	2	0	0·596
	7	11	2	1	0·572
	8	9	2	0	0·4
	9	7	0	0	0·555
Total for D	9	137	16	3	0·452 (average)
F	1	15	4	0	0·388
	2	13	6	3	0·45
	3	13	3	1	0·461
	4	11	2	2	0·545
	5	11	0	1	0·545
	6	10	2	1	0·45
	7	7	4	0	0·476
	8	7	0	0	0·357
	9	4	0	0	0·3
Total for F	9	91	21	8	0·452 (average)
G	1	12	0	3*	0·4
	2	14	2	0	0·364
	3	11	0	0	0·472
	4	11	0	0	0·49
	5	10	0	1	0·54
	6	10	0	1	0·54
	7	9	2	0	0·4
	8	7	2	0	0·514
Total for G	8	84	6	5	0·421 (average)
H	1	20	4	1	0·333
	2	18	2	1	0·394
	3	17	2	0	0·415
	4	16	9	3	0·406
	5	13	6	2	0·476
	6	11	2	6*	0·545
	7	12	6	2	0·472
	8	10	4	0	0·52
	9	9	3	0	0·37
Total for H	9	126	38	15	0·421 (average)

If we pick out from Table I the whorls in which the proportion of com-crescent sporangiophores is highest and arrange them in order of decreasing relative prevalence of concrescence we get the following result:

TABLE II

1. Cone H, whorl 4; 9 out of 16 (or 0·562) sporangiophores concrescent;
      space per sporangiophore: 0·406 mm.
2.   „   H,   „   7; 6 out of 12 (or 0·5) sporangiophores concrescent;
      space per sporangiophore: 0·472 mm.
3. { „   H,   „   5; 6 out of 13 (or 0·461) sporangiophores concrescent;
      space per sporangiophore: 0·476 mm.
    { „   F,   „   2; 6 out of 13 (or 0·461) sporangiophores concrescent;
      space per sporangiophore: 0·45 mm.
4. { „   H,   „   8; 4 out of 10 (or 0·4) sporangiophores concrescent;
      space per sporangiophore: 0·52 mm.
    { „   B,   „   7; 4 out of 10 (or 0·4) sporangiophores concrescent;
      space per sporangiophore: 0·35 mm.
5.   „   B,   „   6; 4 out of 11 (or 0·363) sporangiophores concrescent;
      space per sporangiophore 0·43 mm.

while if we take the whorls in which there are *no* sporangiophores showing any considerable concrescence we get the following result:

TABLE III

1. Cone D, whorl 1; o sporangiophore concrescent out of 25;
      space per sporangiophore: 0·25 mm.
2.   „   H,   „   3; o sporangiophore concrescent out of 17;
      space per sporangiophore: 0·415 mm.
3.   „   B,   „   1; o sporangiophore concrescent out of 16;
      space per sporangiophore: 0·329 mm.
4.   „   D,   „   5; o sporangiophore concrescent out of 15;
      space per sporangiophore: 0·6 mm.
5.   „   D,   „   2; o sporangiophore concrescent out of 13;
      space per sporangiophore: 0·405 mm.
6. { „   B,   „   5; o sporangiophore concrescent out of 12;
      space per sporangiophore: 0·44 mm.
    { „   G,   „   1; o sporangiophore concrescent out of 12;
      space per sporangiophore: 0·4 mm.
7. { „   F,   „   5; o sporangiophore concrescent out of 11;
      space per sporangiophore: 0·545 mm.
    { „   G,   „   3; o sporangiophore concrescent out of 11;
      space per sporangiophore: 0·472 mm.
    { „   G,   „   4; o sporangiophore concrescent out of 11;
      space per sporangiophore: 0·49 mm.
8. { „   G,   „   5; o sporangiophore concrescent out of 10;
      space per sporangiophore: 0·54 mm.
    { „   G,   „   6; o sporangiophore concrescent out of 10;
      space per sporangiophore: 0·54 mm.
9. { „   D,   „   9; o sporangiophore concrescent out of 7;
      space per sporangiophore: 0·555 mm.
    { „   F,   „   7; o sporangiophore concrescent out of 7;
      space per sporangiophore: 0·476 mm.
    { „   F,   „   8; o sporangiophore concrescent out of 7;
      space per sporangiophore: 0·357 mm.

From these tables several conclusions can be drawn. Firstly, it is clear that there is no such correlation as one might expect to find between the crowding together of the sporangiophores in a whorl of the mature cone and the intensity of the tendency to concrescence of sporangiophores. Indeed, if we take the 13 whorls in which the space available per sporangiophore is largest, from 0·6 mm. downwards to 0·514 mm. (D5, D6, D7, D9, F4, F5, H6, D4, G5, G6, B4, H8, G8), we find that out of 145 sporangiophores, 22 are to a considerable extent concrescent with others; while if we take the 16 whorls in which the space available per sporangiophore is smallest, from 0·25 mm. to 0·4 mm. (D1, B8, F9, B1, H1, B7, D2, G2, H9, F1, B6, H2, B3, D8, G1, and G7), we find that out of 213 sporangiophores 33 are to a considerable extent concrescent. The proportion of concrescence is, in fact, not very different.

Again, if we take the seven whorls with the highest proportion of concrescent sporangiophores and arrange them as in Table II in order of decreasing proportion of concrescence, we find that in the first four whorls of this series the extent of space available per sporangiophore is neither specially high nor specially low, being from 0·406 mm. to 0·476 mm.; that in the next two whorls (which show an equal proportion of concrescence) one has a relatively large (0·52 mm.) and the other a relatively small (0·35 mm.) amount of space available per sporangiophore; and that in the last whorl of the seven the amount of space, while not specially small, is inclined to be smaller than most of the medium-sized spaces (0·43 mm.). Finally, if we calculate the average extent of stelar circumference available per sporangiophore for the 43 whorls belonging to five cones included in Table I, we find that it is 0·421 mm., while for the 16 whorls without any and the 7 whorls with the highest proportion of concrescent sporangiophores the corresponding figures are respectively 0·431 mm. and 0·441 mm. Obviously, then, the crowding of the sporangiophores in the mature cone does not go with an increase of concrescence. When we remember how closely packed the sporangiophores invariably are in the young cone this fact is, perhaps, not as surprising as it seems at first sight. Concrescence of sporangiophores clearly takes place very early in the ontogeny of the cone, at a stage at which the sporangiophores are always as closely imbricated as possible (cf. Goebel, 1930, pp. 1253–7). A consideration of this point and of the appearance of the young cone suggests that an important—perhaps the most important—factor producing concrescence of sporangiophores is early vigour of growth manifesting itself especially in the thickness of the stalks. We do not normally find sporangiophores concrescent only by their heads, although the width of these more than compensates for the increased amount of space available at the distal ends of the sporangiophores. Often concrescent sporangiophores are united only by their stalks; and not infrequently only by the proximal parts of the latter. More rarely, though fairly frequently, the concrescence extends to the head, though even then the original members are nearly always distinguishable by the lobing of the complex formed by the concrescent heads. The shortness and stoutness of the stalks of the

sporangiophores of *E. kansanum* no doubt facilitates the spreading of concrescence from the proximal to the more distal parts.

In the cones of *E. kansanum* the highest number of sporangiophores was found in the lowest whorl,[1] the only exception being cone G.[2] As regards the prevalence of bifascicular sporangiophores in *E. kansanum*, although the observations are not yet numerous enough to put the matter beyond doubt, it looks, to judge from Table I, as if the prevalence of a high proportion of these were accompanied by what may be called an erratic decrease in the number of sporangiophores. As already mentioned, the number of sporangiophores in a whorl tends to decrease passing upwards in the cone, though not, of course, at every whorl. By an erratic decrease of sporangiophores I mean one that is not maintained at the whorl above—i.e. cases in which the decrease has presumably outstripped that which is characteristic of the rise in level in the cone. It is interesting to note that the only whorls in Table I in which the number of sporangiophores is less than in the whorls immediately above them are whorls 2 and 4 of cone B; whorl 1 of cone G; and whorl 6 of cone H. These whorls, marked with an asterisk in Table I, are four of the five showing the highest proportion of bifascicular sporangiophores, viz.: 6 out of 11 in whorl 6 of cone H; 5 out of 11 in whorl 4 of cone B; 3 out of 12 in whorl 1 of cone G; and 3 out of 13 in whorl 2 of cone B. The only other whorl in which the proportion of bifascicular sporangiophores is at all comparable to these proportions is whorl 2 of cone F, where it is 3 out of 13, i.e. equal to the lowest of the pro-
..portions quoted.

In 1923 three kinds of anomalous traces found in cones of Equisetum were described. They were found to occur chiefly in the lowest whorls of the cone of *E. maximum* Lam., but were not confined to that position or to that species (Browne, 1923). In the case of the first or A-type of anomaly the phloem of the incoming trace joins up with that of the axial bundles and the tracheides (which in the traces of the sporangiophores of *E. maximum* are normally all of them protoxylem) penetrate into the stele but, failing to connect with the axial protoxylem, die out among the metaxylem elements of the bundle. In the second or B-type of anomaly the phloem of the incoming trace unites with that of the axial bundle, but the tracheides do not penetrate into the latter, though they usually approach it closely. In the third or C-type of anomaly a whole trace passing in from a sporangiophore dies out in the cortex without any of its tissues joining on to the corresponding tissues of the stele. Such traces, also called free traces, die out at very varying depths in the cortex.

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[1] This is true, too, of the other species of the genus so far studied, except *E. silvaticum* and *E. palustre*, in which the sporangiophores were more numerous in the third and fourth than in the lowest whorl (cf. Browne, 1912, Text-figs. 3 and 4, pp. 671–2; Browne, 1921, Text-figs. 1 and 2, p. 431; and Browne, 1933, Text-fig. 3, p. 465).

[2] In this cone there seems to have been a check to the number of sporangiophores initiated in the lowest whorl; for in other cones of *E. kansanum* the number of sporangiophores in the lowest whorl was found to be equal to or slightly greater than the number of bundles and strands of protoxylem in the axis bearing the cone, while in cone G axial bundles and their protoxylem strands numbered 14, and there were only 12 sporangiophores.

Indeed, where a sporangiophore is bifascicular one of the bundles may die out in the stalk of the latter.

So far the B-anomaly has not been found in *E. kansanum* and, except in cone H, the A- and C-anomalies seem to be rare in this species. Neither of them was found in cone D, while in each of the cones B, F, and G there was but one anomalous trace. In cones F and G it was of the A-type and in cone B of the C-type. In cone H there were numerous anomalous traces. Three of them were of the C-type and 21 of type A. Eight of the latter, however, occurred above the ninth whorl where the axial protoxylem was no longer continuous. A special form of the A-anomaly was commoner in cone H than the ordinary type. In it the metaxylem of the incoming trace joins up with the metaxylem of the axis, but the protoxylem dies out without entering the axial bundle.[1] This modified type of A-anomaly seems to be intermediate between the A-type previously described and the B-type of anomaly.

It is by no means rare for a free trace to become markedly wider before dying out in the cortex.[2] This appears to be the result of the slowing down of the differentiation of tracheides before this process ceases altogether. The cells that become converted into tracheides later naturally tend to be larger. In these traces the xylem clearly differentiates inwards from the sporangiophore; and this would seem to be almost necessarily true also of the protoxylem of the traces showing the ordinary A- or the B-type of anomaly. Such a direction of lignification may be a peculiarity of these anomalous traces; but this seems unlikely. Or there may be some variety in regard to this character in the different species of Equisetum for it has been asserted that in the sporangiophores of *E. palustre* differentiation of the protoxylem takes place from the axis outwards.[3]

IV. THE COURSE OF THE VASCULAR STRANDS IN THE CONE

Although the five cones of *E. kansanum* of which reconstructions of the vascular system have been made show a wide range of variation, yet they are all characterized by the presence in the stele of a number of relatively extensive tracts of parenchyma. The extension is chiefly in a vertical direction, though not infrequently the passing out of a whole but narrow vascular bundle as a trace leads to a sudden and marked lateral extension of the parenchyma, two tracts of parenchyma, previously distinct, becoming confluent. In cone F one and in cone H two tracts that arise a little above the departure of the traces of the uppermost whorl of leaves pass through the

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[1] In the figures such traces are shown thus ⊖ and the ordinary A-type of anomaly thus ○.

[2] Such an enlargement has been recorded for some of the free traces of the sporangiophores in *E. maximum* (Browne, 1923, p. 599); and Professor Halle (1936) has described what seems a comparable enlargement of the leaf-traces of the Lower Devonian fossil form, *Drepanophycus spinaeformis* Goepp. (= *Arthrostigma gracile* Dawson). In these fossils, however, the traces are in connexion with the stele and die out towards the periphery of the cortex.

[3] Barratt, 1920, pp. 222–3, Text-figs. 18 and 19. Dr. Barratt's figures are not, however, quite convincing. In Text-fig. 18, for example, the sporangiophores are drawn as though they were, in the greater part of the cone, accurately superposed in successive whorls.

region of insertion of the annulus, through the whole cone, and persist as part
of the parenchymatous tissue which in each of these cones surrounds the
strands running into the apex. In cone G a tract of parenchyma that arises a
little above the level of the last whorl of leaves and in cones B and D similar
tracts that arise near the level of insertion of the annulus extend upwards to
the level of the fertile whorl just below the apical group of sporangiophores.
Besides these tracts of parenchyma we find in all five cones others which,
though less long, extend into as many as five or more (in one case into nine)
pseudo-internodes. Further, we find in all five cones and relatively frequently
in cone H tracts of parenchyma that are confined to a single pseudo-internode;
and others confined to two pseudo-internodes.[1] Despite the long, relatively
slender unbranched strands that are so marked a feature of the reconstructions
of steles of the cones of *E. kansanum* there seems to be some anastomosis in
the neighbourhood of all the pseudo-nodes of the five cones of which recon-
structions of the vascular system have been made.[2]

We also see in each of the five reconstructions of the steles of cones (Figs.
1–5, pp. 428, 429, 439, 443, 445) examples of relatively wide bands of vascular
tissue arising by the fusion of two or more bundles and extending upwards
through the whole or nearly the whole of a pseudo-internode or even for a
greater distance. In cone B there are no less than five such pseudo-internodal
bands of vascular tissue. One, arising from the fusion of two bundles some-
what above the level of the third whorl, extends upwards through over three
and a half pseudo-internodes (cf. Fig. 1) and at first contains a strand of proto-
xylem from each of the two constituent bundles. Near the level of each of
the two next pseudo-nodes a strand of protoxylem passes out in its entirety as
part of a trace; but the number of protoxylems is restored, in the first case by
the branching of the persistent strand, in the second by the fusion of the
vascular band with a branch from a neighbouring bundle, this branch bring-
ing with it its own strand of protoxylem. The other bands of vascular tissue
found in cone B do not extend into more than a single pseudo-internode and
some are definitely shorter than this. In the largest cone, cone D (Fig. 2),
there are four or five pseudo-internodal bands of vascular tissue, all confined
to a single pseudo-internode. In each of cones F and G there seem to be only
two such bands that extend into more than one pseudo-internode, the others
being shorter than this. Three of the four are clearly bivalent; the fourth,
that arising slightly above the third whorl of cone F (cf. Fig. 3), results from
the junction of three strands.[3]

[1] The terms pseudo-node and pseudo-internode are used to designate respectively the
level of insertion of a whorl and the interval between two whorls of the cone. These regions
correspond to the nodes and internodes of the stem; but their homology remains an open
question.

[2] The anastomoses which appear to be associated with the presence of the sixth whorl of
cone H are, however, found somewhat below the departure of the traces.

[3] It breaks up again into three bundles. Though each of the original bundles gives off a
trace the complex is responsible for supplying traces to only two sporangiophores, as two of
the departing bundles enter a bifascicular sporangiophore single in nature.

In cone H there are factors which make it very difficult to estimate the height of the relatively wide vascular bands in terms of pseudo-internodes.
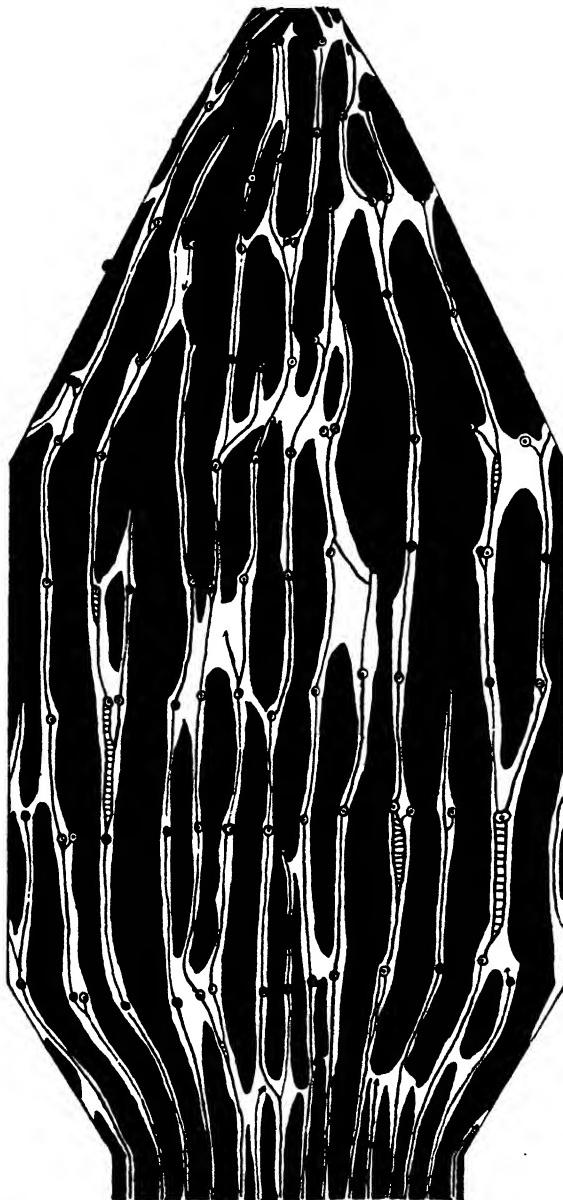
FIG. 3. Reconstruction of the stele of cone F, × 12. For description, see Fig. 1, p. 428.

Besides the irregular course of the strands, which anastomose at very varying levels, complications may arise owing to the occurrence of bifascicular sporangiophores, single in nature; and to the prevalence, particularly in

certain areas, of concrescent sporangiophores. Occasionally, too, the traces of adjacent sporangiophores inserted at nearly the same level on the axis depart from the stele at markedly different levels.[1]

Nevertheless, pseudo-internodal bands of vascular tissue corresponding to more than one bundle are an obvious feature of the reconstruction of the stele of this cone. Table IV gives an analysis of the number of branchings and fusions of both the metaxylem- and the protoxylem-systems, and shows the proportions that these anastomoses bear to the number of sporangiophores present. From it we see that in this character, too, *E. kansanum* has a wide range of variation.

As in the cones of other species the metaxylem is much more freely anastomotic than the protoxylem, and there is no obvious correlation between the branching of the two systems. In the cones studied it would seem that those with fewer sporangiophores showed a higher proportion of anastomosis in both systems. But we cannot establish any definite correlation between the vigour of anastomosis and an increase or decrease in the number of sporangiophores. Similarly, though for both systems the stele with the lowest proportion of anastomosis is that of cone C, and though the higher proportions of anastomoses of both systems are found in cones F and G, yet it is not possible to arrange the steles of the cones in a series showing progressively greater or less frequency of anastomosis of both systems, since cone H, for example, has a much less freely anastomotic metaxylem system than cone B, while its protoxylem system is much more freely anastomotic.

Even when the stele increases in width the number of sporangiophores in the whorls tends to diminish as we pass upwards through the cone. More or less *pari passu* with this diminution, but not quite regularly, a reduction takes place in the number of the axial bundles and their strands of protoxylem.[2]

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[1] These irregularities are particularly noticeable at the level of the sixth whorl of cone H. In this whorl the first sporangiophore is single but has a bifascicular trace; the second and third sporangiophores are single and have normal monofascicular traces; the fourth and fifth sporangiophores each have a normal monofascicular trace but are themselves concrescent; the sixth sporangiophore is single in nature but has a bifascicular trace, one bundle being of the C-type and the other showing the modified A-anomaly. The seventh sporangiophore is also single in nature, but its trace originates as a deeply bilobed structure, both parts showing the modified A-anomaly the eighth sporangiophore is single in nature and bifascicular, both bundles arising from the same axial strand and in contact with one another and both showing the A-anomaly. The ninth sporangiophore is single in nature and bifascicular, both bundles departing from the same vascular strand, but from very different levels; the first (showing modified A-anomaly) passes up for a height of about 300 μ before entering the sporangiophore, while the other originates nearly 400 μ above the first and is somewhat deflected in its course through the cortex, entering the stalk of the sporangiophore at a slightly higher level than did the first bundle (this sporangiophore is situated but little above the higher sporangiophores of its own somewhat irregular whorl and stands much lower than any except the tenth sporangiophore of the next whorl, there being only a height of about 320 μ between the upper and lower surfaces of the stalks of these two sporangiophores, apparently belonging to different whorls). The tenth sporangiophore of the sixth whorl is single in nature and has a normal monofascicular trace. The eleventh and last sporangiophore though single in nature has a bifascicular trace of which one bundle shows the modified A anomaly.

[2] Where bundles have become united into vascular bands the constituent members of the band or complex are, of course, considered in this generalization as bundles.

TABLE IV

Anastomoses of the Vascular Strands in Five Cones of E. Kansanum

Cone.	Number of sporangiophores.*	Metaxylem system.				Protoxylem system.			
		Branchings.	Fusions.	Total anastomoses	Proportion of anastomoses per sporangiophore.	Branchings.	Fusions.	Total anastomoses	Proportion of anastomoses per sporangiophore.
D.	137	12	25	37	0·25	5	2	7	0·051
H.	126	17	18	35	0·25	11	0	11	0·087
B.	94	20	21	41	0·43	6	0	6	0·063
F.	91	26	27	53	0·58	8†	1	9	0·098
G.	84	21	23	44	0·52	9	3	12	0·142
	Total for 5 cones				Average for 5 cones	Total for 5 cones			Average for 5 cones
	532	96	114	210	0·4	39	6	45	0·088

* In *E. kansanum* the sporangiophores in the apical group are at maturity rather more differentiated than in the other species studied, and it is usually possible to ascertain their number. In the above table these sporangiophores have nevertheless not been counted, in order that the figures and facts should be more truly comparable to those ascertained for other species.

† In two cases these branches failed to connect with the parent strand, i.e. the lignification of the elements was at the point of junction delayed long enough for them to develop as metaxylem.

Occasionally the reduction in the number of axial protoxylems is effected by their fusion; but such fusions are much rarer than branchings (cf. Table IV). Still more rarely as, for example, between the third and fourth whorls of cone F, an axial strand of protoxylem dies out in a pseudo-internode (cf. Fig. 3). In the great majority of cases, however, the decrease in number of the axial protoxylems is effected by one of them passing out in its entirety as part of a trace. In the case of the metaxylem system, on the other hand, reduction in number of the bundles is frequently brought about by their fusion in pairs, the resulting strand not forking again a little higher up; or by several bundles becoming united through an increase of their metaxylem—as often occurs in the neighbourhood of a pseudo-node—and the complex thus formed later breaking up into a smaller number of bundles. A glance at Figs. 1–5 shows that reductions in the number of bundles by fusions are very often associated with passings out in their entirety of protoxylems as parts of traces from one of the fusing bundles. In other cases, particularly where the number of bundles in the axis is being rapidly reduced, the whole of one or more narrow bundles may pass out as a trace. In such cases the traces appear in the diagram to be terminal.

If we consider the incidence of these two contrasted methods of reduction in number of the bundles in the cones of those species of Equisetum of which complete reconstructions of the steles of the cone have been made, we find that, apart from cases occurring near the apex of the cone, the passing out of an axial bundle in its entirety as a trace does not seem to occur in *E. maximum*, *E. arvense*, and *E. palustre*; that it is very rare in *E. silvaticum*, where it seems to occur chiefly in the three to four whorls immediately below the apical group of incompletely differentiated sporangiophores; and that it occurs occasionally in *E. giganteum* and *E. hiemale*. *E. kansanum* shows a somewhat stronger tendency for the reduction in number of bundles to be effected by this method than do these cones; but in it this tendency is not nearly so marked as in *E. debile* or as in the larger of the two cones of *E. limosum* of which reconstructions of the stele of the cone have been made; in the smaller of these two cones cases of the passing out of the whole bundle as a trace were observed, but they were not numerous.[1]

The absence in the cone of *E. maximum* of vascular bundles that pass out in their entirety as traces might seem surprising, since in this species the number of sporangiophores and bundles decreases very greatly as we pass up the cone. But in *E. maximum*, *E. arvense*, *E. palustre*, and *E. silvaticum* the xylem of the traces of the sporangiophores consists entirely or almost entirely of protoxylem; and when the whole of an axial strand of protoxylem departs in a trace, the metaxylem remaining in the axis usually fuses with a neighbouring bundle provided with a strand of protoxylem.

One of the interesting points about *E. kansanum* is that it shows almost every conceivable transition between these two apparently sharply contrasted methods of reduction in number of the bundles in the axis of the cone. Thus, at each of the levels of the seventh and eighth whorls of cone G (cf. Fig. 4) we see a bundle pass out in its entirety as a trace; while in each of the fifth, sixth, and seventh whorls of the same cone a sporangiophore receives a trace which carries off the whole of the protoxylem and nearly all the metaxylem of the bundle from which it is given off, the few tracheides of the latter remaining in the axis dying out a few sections higher up.[2] In other cases in which nearly all the metaxylem passes out with the protoxylem as part of the trace, the tracheides left in the axis, though no more numerous than in the two examples just mentioned, persist, pursue a steeply oblique course, and fuse with an adjacent bundle. This occurs after the departure of the third trace of the second whorl of cone G, and in whorls 2 of cone D and 5 of cone

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[1] Browne, 1912, 1915, 1920, 1921, 1933. In cones of *E. variegatum* the conditions are hardly comparable. There are usually but four or five whorls and the decrease in the number of sporangiophores in the whorls as we pass up the cone is very small—often only from seven to six or to five. Generally all the bundles, except the one that penetrates the strongly vascularized apical point, pass out in their entirety as traces of the *uppermost* whorl. Cf., however, Browne, 1921, Text-fig. 8, p. 436 for a strand passing out in its entirety as a trace of the whorl below the uppermost one.

[2] In the diagram such metaxylem elements seem to form a little cap above the trace.

H, towards the middle of the diagram. By searching the reconstructions (Figs. 1–5, pp. 428, 429, 439, 443, 445) the reader will be able to observe cases which can be so arranged as to form a series in which the amount of metaxylem
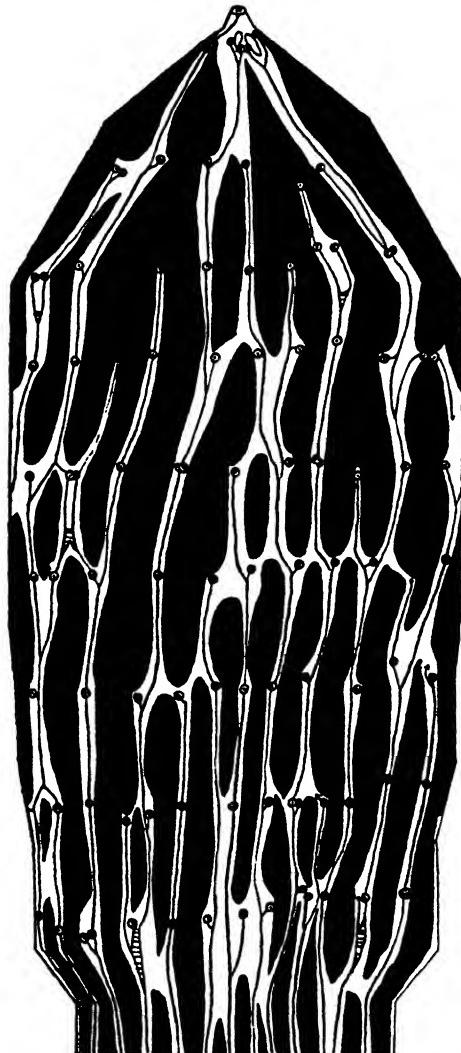
FIG. 4. Reconstruction of the stele of cone G of *E. kansanum*, × 12.
For explanations see Fig. 1, p. 428.

left after the departure of the trace is increasingly greater and greater, until the two fusing bundles are of equal size, though one of them has parted with the whole of its protoxylem to a trace and the other has not. The fusion of two whole bundles and of their strands of protoxylem may occur, but is very rare.

To sum up: except, perhaps, in *E. debile*, reduction in number of the

bundles of the axis of the cone is more often effected by the fusion of the bundles than by their dying out or their passing out in their entirety in traces. In the protoxylem system, however, fusion of axial strands is either very rare or does not occur. The axial strands of protoxylem occasionally die out in the axis itself; but in the great majority of cases their reduction in number is due to their passing out in their entirety as part of traces. Consequently the fusion of a bundle devoid of a strand of protoxylem with a neighbouring one possessed of such a strand is very common.

A tendency to discontinuity of the protoxylem can be observed in cone H. This is most marked in the region above the ninth whorl, where the six remaining strands of axial protoxylem are, as it were, broken up into short vertical lengths. This results from delayed vascularization of some of the cells, these becoming converted into tracheides at the same time as the typical metaxylem, by which time they have attained the same size as the cells of the latter. There are in this cone two cases of a longer break in continuity of the axial protoxylem. The first occurs just above the departure of the trace of the fourth sporangiophore of the sixth whorl. Here the narrow bundle from which the trace has departed contains for about 500 μ no protoxylem; but this then reappears and the protoxylem strand thus formed subsequently gives off a trace to a sporangiophore in each of the next three whorls. The second case occurs between the third and fourth whorls. Here the fourth bundle from the edge of the diagram on the reader's right, arising from the fusion of two strands at the level of the third pseudo-node, has but one protoxylem strand, that of the second bundle having passed out into a sporangiophore of the third whorl. About half-way up the pseudo-internode this protoxylem strand appears to swerve to the reader's right. Actually, a study of the specimen shows that branching of the protoxylem would have occurred at the level of the swerving had not the vascularization of the elements of one branch of the fork been delayed, so that on differentiation these developed as metaxylem. As a result not only is the protoxylem of the bifascicular trace of the twelfth sporangiophore of the next (fourth) whorl not in connexion with any axial protoxylem, but there is no such strand opposite to or nearly opposite to the incoming trace, the axial protoxylem strands being in this region from one and a half times to twice as far apart as the others at this level. One of the bundles of this bifascicular trace dies out in the cortex without entering the stele; but above the other, protoxylem elements are again differentiated; and though these are at first broken up by intervening metaxylem elements, they soon come to constitute a continuous axial strand of protoxylem which runs through three pseudo-internodes before departing in its entirety as part of a trace.

These cases of delayed differentiation of tracheides afford a parallel to the traces showing the A anomaly—in their case the delay affects, not the vascular branchlet destined to the trace, but portions of a main axial strand of protoxylem of the cone.
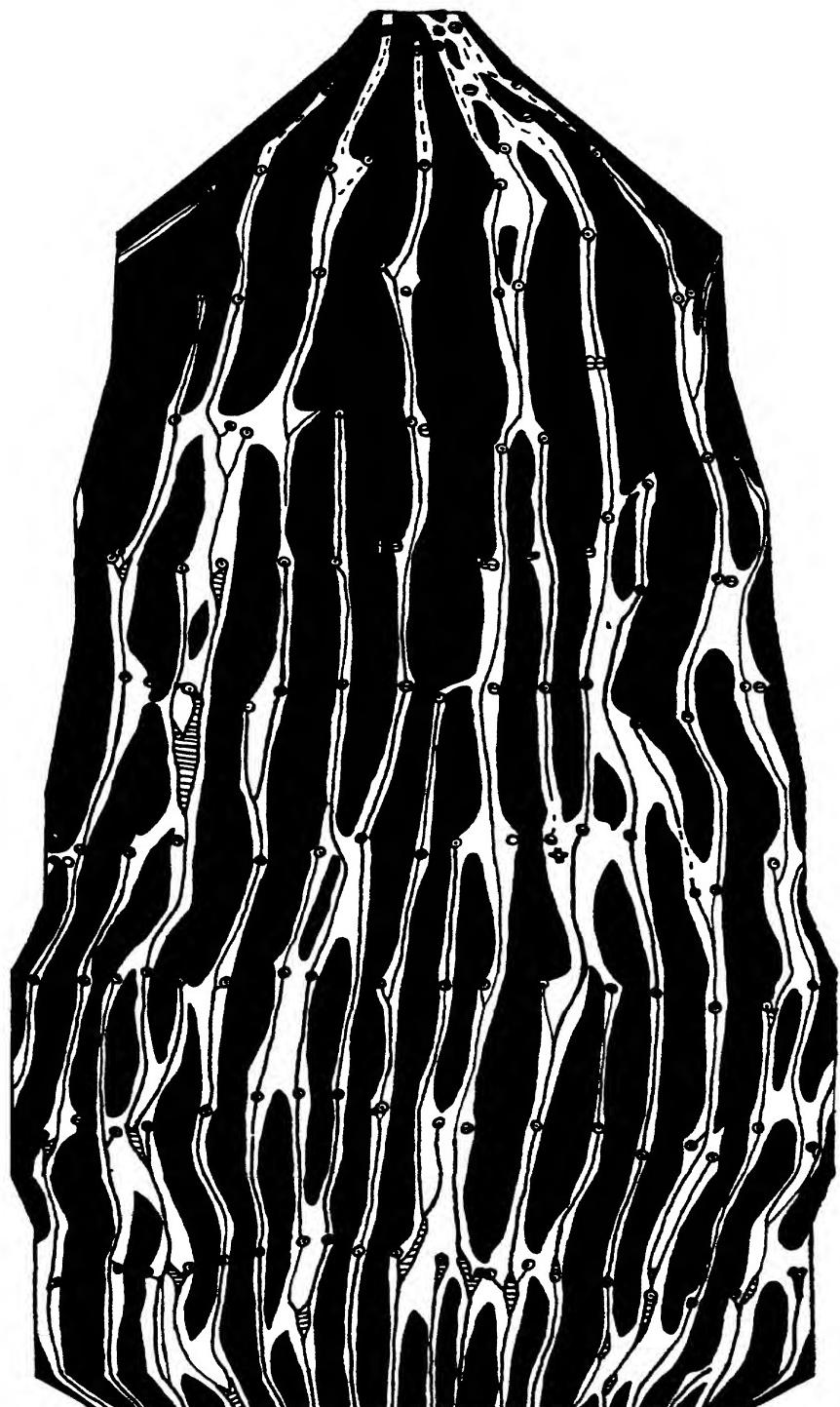
FIG. 5. Reconstruction of the stele of cone H of *E. kansanum*, × 12.
For explanations see Fig. 1, p. 428.

V. The Anatomy of the Axis in the Region of the Annulus

As the level of insertion of the annulus is approached the narrow internodal bundles of the stem widen, while at or near this level—usually just above it— the metaxylem-tracheides increase in number and, instead of forming two parallel radial rows, arrange themselves in a not very regular transverse band, one or two (locally more) cells deep. At the base of the cone the small carinal canals are usually persistent.

In cones D, B, and F, of *E. kansanum* the anastomoses of the axial bundles in the neighbourhood of the insertion of the annulus are relatively numerous. In cone D, where below the annulus the axis contained twenty-three vascular bundles, no less than twenty fusions and eighteen branchings occurred. In no other species so far studied, not even in *E. hyemale*, have so many anastomoses of the vascular bundles been recorded, regard being had to the number of bundles present. Below the annulus of cones B and F of *E. kansanum* the axis contained fifteen bundles, and in both cases there were nine examples of fusion and seven of branching; while in cones G and H, where the number of bundles was respectively fourteen and eighteen, there were in the former four cases of fusion and four of branching; and in the latter six of fusion and four of branching.

As in the other species studied the protoxylem system in this region as also in the cone proper, was much less freely anastomotic than the metaxylem-system. In cone D there were two cases of branching and one of fusion of protoxylems, while in each of the cones B, F, G, and H, there were no fusions of protoxylems and but one or two cases of their branching.

VI. Discussion

Two conflicting views of the phylogeny of the vascular system of the cone of Equisetum have been suggested. On the one view that system is held to be derived from a siphonostele; on the other from a circle of separate strands running vertically upwards without anastomoses, except such as are due to a change in the number of bundles. The first view was put forward by me in 1912 as a result of a study of the cones of *E. arvense*, *E. palustre*, and *E. limosum* (Browne, 1912). Subsequent work on the cones of *E. maximum*, *E. hyemale*, *E. giganteum*, *E. sylvaticum*, *E. debile*, and *E. variegatum* (Browne, 1915, 1920, 1921) did not at first shake this view, which was supported, though on different grounds, by Dr. Barratt (1920). The second view was put forward in 1933, the choice between the two views being left completely open.[1]

In the light of the wide variation exhibited by the reconstructions of the vascular systems of cones in Figs. 1–5 it is no easy matter to say which of these alternative views receives support from a study of the cone of *E. kansanum*.[2]

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[1] For a short statement of both views the reader is referred to Browne (1933), pp. 469–74.

[2] Since in no other species have complete reconstructions of the stele been made, for so many specimens this result affords a useful warning against generalizations about the degree

To my mind the vascular systems of the cone of this species are on the whole suggestive of a stele of separate strands, occasionally anastomosing and being thus brought more nearly opposite to the sporangiophores which they supply with traces,[1] than of a modified siphonostele. Nevertheless, the stele of the cone of *E. kansanum* is by no means as suggestive as that of the cone of *E. debile* of a vascular system that has arisen from a circle of separate strands (cf. Browne, 1921, pp. 433–5 and Text-figs. 3–8). In *E. kansanum* the impression results chiefly from the number of axial bundles with a single strand of protoxylem that remain narrow and pursue an unbranched course through several pseudo-internodes, giving off traces to but one sporangiophore of each whorl.[2] Such bundles are especially common in cones H and D, and are a marked feature of cones B and F. In the smallest cone, cone G, they are also to be found, but they are less noticeable; and at the levels of some of the fertile whorls, particularly of the second and fourth, the anastomosis is sufficiently marked to suggest the possibility that in a specimen with rather more metaxylem there might be at these levels a ring of xylem.

On the other hand, it must be admitted that if we rejected the siphonostelic origin of the central cylinder in the cone of Equisetum it is difficult to give an adequate explanation of the bands of pseudo-intervascular tissue extending through one or more pseudo-internodes and consisting of two or more united vascular bundles, unless they are regarded as vestiges of a siphonostele. Such bands of vascular tissue are found frequently in cones of *E. arvense* and of *E. maximum*, and perhaps, particularly frequently in those of *E. giganteum* and *E. kansanum*; they occur also in cones of *E. hyemale* and more sparingly in cones of *E. sylvaticum*, *E. palustre*, and *E. limosum*. It has been suggested that the tendency, seen in *E. arvense* and *E. maximum*, for the metaxylem to decrease radially and spread laterally facilitates the formation of such bands. But this tendency does not exist in *E. giganteum*, where the metaxylem is particularly well developed radially, nor in *E. kansanum*, though these bands are freely developed in the cones of both these species. It has also been suggested that the acquisition of alternation by the sporangiophores, probably towards the close of the Palaeozoic or the beginning of the Mesozoic era, may have led to vigorous anastomosis of the bundles at the level of the fertile whorls and that the close imbrication of the sporangiophores of successive whorls that followed their alternation has led to a marked shortening of the distance between the whorls, so that the increased metaxylem has in sectors of the stele come to extend through one or more pseudo-internodes (Browne,

of anastomosis that is characteristic of the vascular system of the cones of different species. It should, however, be remembered that in the species investigated the stele was studied in several cones and the reconstructions made were believed to be of typical vascular systems; and also that the question with which we are now concerned is not what degree of anastomosis is characteristic of the steles of the cones of different species, but which are the more primitive systems and whether these have been derived from a siphonostele or from a ring of bundles.

[1] The commonest method of adjustment to this task in this species seems, however to be a divergent course of the trace through the cortex (cf. Browne, 1933, pp. 467–78).

[2] Owing to precocious division some of these traces may be bifascicular.

1933, pp. 470 and 472). On broad anatomical grounds it is safe to say that the tendency noted in the present paper (cf. pp. 427–30) for a decreasing amount of metaxylem to pass out in the trace of a sporangiophore is certainly a derivative character. We have seen, too, that the effect of this retention of additional metaxylem in the axis has been sufficient to alter the method of the reduction in number of the axial bundles and therefore to modify somewhat the nature of the stele (cf. p. 442). The retention of this metaxylem in the axis may have been a factor facilitating the formation of the vascular bands under consideration. This argument, again, cannot be used to explain the prevalence of these bands in *E. giganteum* and *E. kansanum*, in both of which the traces carry off a relatively large number of metaxylem-tracheides from the bundles. When these bands of vascular tissue occur near the apex of the cone they may be partly explained by the greater reduction in size of the stele, proportionately to the reduction of the number of axial bundles.

Some light is thrown on the effect of reduction in size of the cone by Professor M. Johnson's (1933) account of *E. scirpoides* Michaux, the cones of which are the smallest in the genus, seldom exceeding 10 mm. in length and consisting of three or four whorls of three members each, surmounted by a cap of sterile incompletely fused sporangiophores. The sporangiophores and their traces in the axis apparently alternate regularly in successive whorls. As the lowest whorl of the cone is approached the metaxylem of the bundles becomes tangentially extended and the bundles tend to fuse, though in the larger cones a gap may remain in the ring of vascular tissue.[1] To judge from Professor Johnson's account these anastomoses seem in *E. scirpoides* to be confined to fusions.[2] In the smaller cones the bundles remain united into a cylinder, reduced above the second whorl to a small rod of xylem, surrounded by phloem; above the third and last whorl of traces no vascular tissue is differentiated. In the larger cone tracts of parenchyma are found, and Professor Johnson, whilst alluding to these as gaps, emphasizes the fact that though they usually arise vertically above the traces, they are not gaps left by the latter.

Both Milde (1867, pp. 660–8) and Dr. Schaffner (1930, p. 102) have maintained that the nearest relative of *E. scirpoides* is *E. variegatum*. Dr. Schaffner holds that *E. scirpoides* probably originated from the ancestral type of *E. variegatum*. If this was so, its cone has undoubtedly undergone reduction both in the number of members in a whorl and in the diameter of the stele; but the reduction in number of the sporangiophores in a whorl from 5–8 in *E. variegatum* (Duval-Jouve, 1864, p. 212; Browne, 1921, p. 436) to three in *E. scirpoides* seems to have been less proportionately than the reduction in diameter of the stele. According to Professor Johnson's Figures 17 and 18—

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[1] These fusions are probably correlated to the insertion of the annulus. Such anastomoses, found in all the cones studied except in that of *E. variegatum*, often occur above the annulus and close to the insertion of the lowest whorl of sporangiophores.

[2] The same is true of the cone of *E. giganteum.*

it is true of the smaller cones—taken from sections above the second and third whorls, the diameter of the vascular tissues was from about 0·06 mm. to about 0·04 mm. while in *E. variegatum* I found, at the same levels, vascular tissue with a diameter of from 0·6 mm. to 0·4 mm. (1921, Figs. 8–10, p. 436). Professor Johnson notes that as the tip of the cone is approached the stele decreases more rapidly in size than do the tracheides in number, so that the gaps become fewer and smaller until they disappear and we get a siphonostele and eventually a solid vascular strand. He adds, however, 'The formation of gaps, then, is dependent not only upon a reduction of xylem as has been generally proposed [*sic*], but upon the failure of the fundamental tissue[1] to reduce in the same proportion (Johnson, 1933, p. 941)'. This sentence seems to imply that parenchymatous gaps in the circumference of the stele were not present before the discrepant reduction came into play. Comparative evidence, however, especially that drawn from *E. variegatum*, indicates that neither in its phylogeny nor in its ontogeny was the stele of *E. scirpoides* derived from a continuous siphonostele. On the contrary, it would seem that, as pointed out above, in its ontogeny as well as in its phylogeny the presence locally of siphonostely or of an approach to it is due to very considerable reduction in size of the stele.[2] It would be possible to hold that a relatively great reduction in size of the stele, coupled with a tendency for the metaxylem to develop laterally rather than radially, explained the tendency to siphonostely exemplified in some cones of *E. arvense*. The vascular system of the cone of this species in which this tendency was most marked was a relatively small one (cf. Browne, 1912, pp. 667 and 669, Figs. 1 and 2. The latter figure has been printed inverted).

In 1935 the late A. Santschi published a paper dealing chiefly with the cone of *E. ramossisimum* Desf.[3] For our present purpose the principal interest of this paper lies in Santschi's Fig. 1 (p. 107), described as showing the course of the bundles in *E. ramossisimum*. This figure is remarkable because it shows an extraordinarily irregular disposition of the departing traces. Santschi remarks that it does not allow of the recognition of whorls of sporangiophores and even militates against our observing sporangiophore-bearing regions, although, owing to mutual pressure, the heads of the sporangiophores arrange themselves so as to be apparently disposed in whorls. He adds that 'nevertheless the distribution of the sporangiophores is not quite haphazard' (p. 111). He recognizes two sporangiophore-bearing regions, one with six the other with seven or eight traces; states that other sporangiophores originate between

[1] Intrastelar fundamental tissue would seem to be the tissue actually influencing the size of the stele and through this the presence of parenchymatous tracts.

[2] The reduction in size of the stele postulated above is at present claimed only for the cone of *E. scirpoides*. Nevertheless it may be significant that while some of the *Equisetites* of the Mesozoic rocks were larger than recent species of Equisetum there seems to be no conclusive evidence that the cones of such species of *Equisetites* were larger than or even as large as some of the cones of *Equisetum maximum*.

[3] Figures and short descriptions of the sporangiophores of *E. maximum*, *E. arvense*, and *E. palustre* were also given.

these two regions.[1]   This distribution of the traces Santschi contrasts with that in the cones described by Dr. Barratt and myself, remarking that though in these latter the verticillate order is more or less disturbed at the apex 'it is undeniable in the rest of the cone' (p. 108). Actually, in the cones studied by me, by far the greatest irregularity in the disposition of the departing traces was found in the *middle* region of a large cone of *E. maximum*. In this region of that particular cone it was often impossible from the position alone of many of the departing traces on the stele to say to what whorl a given trace belonged (Browne, 1915, Pl. XIII).

Santschi tells us that having been unable to obtain a satisfactory result by embedding in paraffin and making serial microtome sections, he was obliged to have recourse to a method involving the use of a solution of collodion and the cutting of sections 'à main levée' by a hand microtome, the sections being afterwards cleared by Eau de Javelle. His diagram apparently represents the lower part and less than half of the whole length of the stele.[2] Unfortunately, neither the dimensions of the cone nor the scale of the reconstruction is given, so that the thickness of the sections can only be approximately estimated. In Santschi's four figures of transverse sections of the axis of the cone of which the magnification is given, the bundles varied from six to eight in number, just as they do in his reconstruction of the vascular system of a cone; and the diameter of the steles varied from three-quarters of a millimetre to one millimetre. If we assume that in the cone on which the reconstruction is based the diameter of the stele fell within these limits, the scale of Santschi's reconstruction would be from 11 to 14 times life-size. On such a basis the width of the bundles in the reconstruction agrees well with the width of the bundles in those figures of Santschi's of which the scale is given. But if a magnification within these limits be accepted for the reconstruction, the hand-microtome sections would be cut at levels from $384\,\mu$ to $489\,\mu$ apart. Even with sections cleared by Eau de Javelle this would almost inevitably lead to the missing of the exact level of departure of some of the traces. Again, if the remaining sections of the cone were cut as far apart as those shown in the diagram, the cone would, on the basis of a magnification of 11 to 14, have attained a length of from $33\frac{3}{4}$ to over 43 mm. In Baker's *Handbook of the Fern Allies* (1887) it is said that the cones of *E. ramossisimum* vary from $\frac{1}{4}$ to 1 inch in length, i.e. from $6\frac{1}{4}$ to 25 mm. in length.[3] An examination of the European material of *E. ramossisimum* in Kew Herbarium confirmed Baker's figures. Numbers of very small cones were noticed, some immature. The

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[1]   In his reconstruction there seem to be five traces departing between these two regions and ten below them.

[2]   The cone was cut up into 88 sections and the reconstruction is said to have been made from sections 51–8, 60–70, and 72–88, allowance being made in it for sections 59 and 91. Since in the diagram the stele does not diminish in width, as it certainly would in the upper part of the cone, the reconstruction must be of the lower part of the stele and the series of sections must have been numbered from above downwards (cf. pp. 107 and 109).

[3]   Milde (1867, p. 431) merely remarks that the cones of this species vary much in size.

larger cones usually attained a length of from 10 to 13 mm.; two or three only attained a length of from 23 to 35 mm.; and these were relatively wide cones. So far as could be seen, this represented the extreme limit of length. Unless, therefore, Santschi's reconstruction was of a quite abnormally long and slender cone, it cannot have been accurately drawn to scale. He claims that his reconstruction was made from diagrammatic but accurate camera-lucida drawings of each section, recording the positions of the protoxylems and the points of departure of the traces. He asserts that in the majority of cases the orientation of the drawings was accurately determinable, but he admits that in some cases these drawings had to be laterally adjusted to obtain their correct positions (1935, p. 108). And in commenting on the apparent increase of metaxylem in the upper part of his reconstruction, he remarks that, apart from the number of bundles having increased 'we are dealing with a projection on a cylinder and not with the real dimensions of the bundles'.[1]

Two of Santschi's sections, reproduced as Figs. 6 and 7 of his paper, add considerably to our reasons for doubting the accuracy of his reconstruction. His Fig. 6, p. 114, representing a section through a complete axis of a cone at the level of insertion of the sporangiophores, shows the traces of seven of the eight sporangiophores,[2] passing out very nearly if not quite radially into these organs, while the appearance of the bundle opposite to the eighth sporangiophore makes it clear that the emission of a trace is either just beginning or has just been completed. Santschi's Fig. 7, p. 115, includes but half the axis and shows the bases of four sporangiophores, two of which are completely concrescent. In it five traces are passing out radially to the sporangiophores, three from a rather wide continuous band of vascular tissue, and two from separate but neighbouring bundles. Both these figures—and they are the only ones in Santschi's paper that show the bases of more than two sporangiophores—are in flagrant contradiction with his description of the highly irregular insertion of the points of departure of the traces on the stele, and with his reconstruction of the stele. On the other hand they, as well as his Fig. 3, p. 109, do bear out one character shown in the reconstruction, namely the tendency for two, sometimes for three, bundles to become united by metaxylem, thus giving rise to bands with two, or sometimes with three, strands of protoxylem. According to the reconstruction these bands with more than one protoxylem-strand persist for a considerable height.

We may now summarize very briefly the effect of recent investigations on the question of whether the irregular and very variable network of vascular strands characteristic of the cones of Equisetum has arisen from a siphonostele or from a circle of separate, vertically continuous strands. The anatomy of *E. kansanum* is, on the whole, suggestive of the latter rather than of the former alternative. If, in the cone of *E. ramossisimum*, the bands of vascular tissue shown in Santschi's figures of transverse sections of the cone really possessed

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[1] '... il s'agit d'une projection sur un cylindre et non des dimensions réelles des faisceaux' (p. 110). [2] Some of these appear to be double in nature.

the vertical extension given them in his reconstruction, this would be a point in favour of the siphonostelic origin of the stele in the cone of Equisetum. Unfortunately, however, there is reason to suppose that the reconstruction is seriously inaccurate in very considerably exaggerating both the irregularity of the points of departure of the traces from the stele and the length of the stele compared to its width. These discrepancies between Santschi's reconstruction of a stele on the one hand and his figures of actual transverse sections of the cone, as well as the hitherto recorded dimensions of the cone of *E. ramossismum* on the other, make it unadvisable to attach much weight to the apparently considerable vertical extension of relatively wide bands of vascular tissue shown in his reconstruction. As regards *E. scirpoides* it would seem that the approach to a siphonostele in the axis of its cone, far from being favourable to the view that the vascular system of the cone has been derived from a siphonostele, tells against such a derivation. For it seems clear that not only has the cone of this species undergone reduction in size, but also that the approach to siphonostely is correlated to and has arisen through the relatively greater reduction in amount of the centrally situated than of the peripheral stelar tissues. The question of the primitive form of the stele in the cone of Equisetum remains open. To my mind the evidence is, on the whole, slightly in favour of the view that this stele has been derived from a circle of separate strands. But, if this be so, it must be admitted that any considerations so far advanced are inadequate, at least in the case of *E. giganteum*, to account for the tendency towards the development of relatively wide bands of vascular tissue, not only at the level of the pseudo-nodes but also in the pseudo-internodes.

SUMMARY

1. In an account of the anatomy of the cone of *E. kansanum* Schaffner it is shown that the stele of this cone is characterized by the presence of an irregular network of bundles. While the five steles of which reconstructions were made showed a wide variation in the degree and nature of this anastomosis, all of them were characterized by the presence of tracts of parenchyma of considerable vertical extent, and of occasional lateral bands of metaxylem corresponding to more than one bundle.

2. In the vascular strands the relation between protoxylem and metaxylem is liable to change from one of contact to one of separation by a few parenchymatous cells. More often than not the metaxylem is in contact with the protoxylem or with the small lacuna that may replace the latter.

3. As is usual in the cones of the genus the protoxylem system is far less freely anastomotic than the metaxylem-system; and there is no obvious correlation between the anastomoses of the two systems.

4. In the bundles of the axis of the cone there is a marked tendency to irregularity in the rate of differentiation of the protoxylem-strands even in adjacent bundles and at the same level. In some protoxylem-strands the conversion of certain elements into tracheides is delayed to such an extent that

they resemble metaxylem and cause discontinuity of the protoxylem over a small vertical distance.

5. The tracheides in the traces of the sporangiophores are relatively numerous, consisting of metaxylem as well as of protoxylem. Evidence was found that in some cases at least the differentiation of the protoxylem of the incoming trace takes place from the sporangiophore inwards.

6. Bifascicular sporangiophores, single in nature, are not uncommon. Lateral concrescence of two or more sporangiophores of the same whorl is relatively common in this species. It was found that there was no correlation between the crowding of the sporangiophores in the *mature* cone and the prevalence of this concrescence.

7. In the region of insertion of the annulus anastomoses of the vascular bundles are relatively frequent, regard being had to the number of bundles present. A few cases (1–3) of anastomosis of the protoxylem strands at this level were found in each of the cones studied.

8. The bearing of these facts and of recent investigations of other species on the question of whether the original form of the stele in the cone of the Equisetaceae was a siphonostele or a ring of separate bundles is discussed.

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Experimental Studies of the Relation between Carbon Assimilation and Stomatal Movement

II. The Use of the Resistance Porometer in Estimating Stomatal Aperture and Diffusive Resistance

Part i. A Critical Study of the Resistance Porometer

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With eleven Figures in the Text

INTRODUCTION

IN a previous paper in this series (Heath, 1939) the apparatus and technique used in following simultaneously in the same leaf changes of assimilation rate and stomatal opening were described. For following stomatal opening the principle of the resistance porometer (Gregory and Pearse, 1934) is used. One of the commonest and most cogent criticisms of the porometer method of following stomatal movement is that it does not enable one to estimate the aperture of the stomata. The desirability of calibrating the porometer in terms of actual stomatal aperture is self-evident, and for the purposes of investigation of the relation between carbon assimilation and stomatal movement calibration in terms of the diffusive resistance of the stomata is even more important. In the present paper (Part 1) the resistance porometer method is critically discussed and its use in estimating stomatal resistance to mass flow of air is considered. Parts 2 and 3 will describe the attempts to calibrate the porometer in terms of stomatal diffusive resistance and stomatal aperture. The problems involved have proved to be of some complexity, and although the investigations are not complete in all respects, it is thought desirable to publish an account of them, both in explanation of the use made of the porometer readings in the assimilation experiments (to be published later) and as a basis for further work, especially as experimental work on the subject has had to be suspended for the time being.

Not many attempts to calibrate porometers have been published. Paetz (1930) made use of a Darwin and Pertz (1911) type of porometer and measured stomatal aperture by direct microscopic observation of the living leaf using a Leitz 'Opakilluminator'. Following a porometer reading ten stomata were measured, presumably on the area of leaf previously occupied by the porometer cup. Expressing both the porometer rate and the stomatal width (*Zea mais*), or half the geometric mean of the width and length (*Tradescantia fluminensis*), as percentages of the maximum values obtained, he compared the observed points with a series of theoretical curves in which the porometer rate was proportional to the first, second, third, &c., powers of the stomatal dimensions. He concluded that for 'not too wide' stomata the porometer rate was proportional to the cube of the stomatal width.

Ashby (1931) working with Pelargonium and Verbena made a comparison of the readings of a Knight (1915) porometer with stomatal areas as measured by Lloyd's (1908) method on strips of epidermis from another leaf. By plotting both sets of data as percentages of the maximum values obtained he showed that the time curves given by the two methods were in good general agreement, although his application of the χ^2 test of significance (Fisher, 1925–38) to the differences of the percentage values was incorrect. Beyond demonstrating this general agreement he made no attempt to calibrate the porometer in terms of actual stomatal aperture.

Newton (1936), in the work at this Institute which gave rise to the present

studies, carried out on *Pelargonium zonale* a preliminary calibration of the resistance porometer in terms of stomatal area as measured by Lloyd's method. Immediately after a porometer reading the cup was rapidly removed from the leaf, and a strip of epidermis was taken from within the area it had covered and plunged into absolute alcohol. Thirty stomata were measured microscopically on each strip. For this calibration Newton made use of only a few leaves at only one time of year, and the results for all the leaves apparently lay on the same curve. He concluded that the resistance to viscous flow was inversely proportional to the 2·76th power of the stomatal area and he gave the necessary constant for the conversion of resistance readings to actual stomatal areas. Preliminary work by the present author indicated that Newton's findings represented an over simplification of the problem and the further investigations to be described later were accordingly undertaken.

With reference to the work of Paetz mentioned above, Williams (1940) has suggested that the empirical result obtained, namely that flow is approximately proportional to the cube of the stomatal width, might be expected on theoretical grounds. If flow through a stoma obeys Poiseuille's formula for viscous flow of a gas through a capillary tube of elliptical section, under given conditions:

$$\text{Vol. of gas per sec.} \propto \frac{a^3 b^3}{a^2 + b^2},$$

where a and b are the semi-axes of the ellipse. Since for small and medium openings a is small compared with b, a^2 is even smaller compared with b^2 and may be neglected in the denominator. Then since b, the long axis of the ellipse, is almost constant, the volume flowing is proportional to a^3. The same argument might perhaps be applied to Newton's finding that the flow is proportional to approximately the cube of the area, for since the long axis is almost constant the flow must also be proportional to approximately the cube of the width. It should be noted, however, that in Newton's results the proportionality between flow and 2·76th power of the area extends down to the largest stomatal apertures when a is not very small compared with b.

Attention may now be turned to previous work on the relation between the resistance or conductance of the leaf for viscous flow as measured by a porometer and the stomatal resistance or conductance for gaseous diffusion. Darwin (1916) considered that the stomata should be treated as long narrow tubes, and assumed that diffusion would vary directly with the stomatal area while viscous flow would be proportional to its square, as for a long capillary tube of circular cross section. He therefore used the square root of the porometer rate as a measure of diffusive conductance. Maskell (1928) also made use of the square root of the porometer rate (rate of flow with a Darwin type porometer but corrected for certain sources of error) as 'the closest simple approximation that can be made to an experimental measure of stomatal diffusive capacity'. Only one attempt to solve this problem experimentally has been

published,[1] that of Gregory and Armstrong (1936) who described a porometer—the diffusion porometer—which for the first time enabled direct measurements of the diffusive resistance of the leaf to be made. They gave for Pelargonium a curve relating readings of the resistance porometer to rates of hydrogen diffusion through the leaf, the hydrogen being measured in terms of the current necessary for its production. These data were subsequently re-examined by Newton (1936) and found to give the hydrogen diffusion inversely proportional to the 4·74th root of the resistance porometer reading. The present author's experiments give results which are not in agreement with this finding, and possible reasons for this disagreement will be discussed in the appropriate context, but there is not doubt that the diffusion porometer provides a method of investigation of the utmost importance which should be developed and refined to the greatest possible extent.

MATERIAL

A few experiments have been carried out with leaves of *Begonia sanguineum* obtained from Chelsea Physic Garden, but for the main part of the work, both for porometer calibration and assimilation experiments, *Pelargonium zonale* has been used as in Newton's (1936) investigations. Newton was not able to obtain plants grown under comparable conditions for his various experiments, and in order to avoid this possible source of error five clones of *P. zonale* (var. Paul Crampel) were started from cuttings in September 1936. Some leaves were used for preliminary porometer experiments in 1937, and two of the clones (No. 3 and No. 5) were repropagated in September 1937 to give a total of fifty plants for 1938. Both these clones were made use of in 1938 for assimilation experiments and for porometer calibration, and No. 5 was repropagated in September 1938 to provide material for 1939. The old plants of No. 3 were kept on and repropagated in June 1939 to provide material for the autumn, since it had been found that leaf size generally fell off seriously in September in the case of autumn propagated plants. Actually both clones were used for porometer experiments in autumn 1939. By 1940 there were sufficient plants of a single clone (No. 5) for both summer and autumn cuttings and future work will be carried out on this clone.

From the time that the cuttings were taken the plants were grown together under the same conditions,[2] being kept under glass in a frame with very slight heating. Watering and repotting were carried out at the same times for all plants; all flower buds were removed as they appeared.

The stomata of Pelargonium plants grown under glass seem to remain actively responsive to the stimulus of light for a much greater part of the year than those of outdoor plants, and the leaves grow to a greater size. Such leaves also have a smaller number of stomata on the upper surface relatively

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[1] Maskell (1928) quotes unpublished work on the subject by Briggs and Maskell.

[2] Some of the plants were removed to a similar frame at Rothamsted Experimental Station for use in porometer experiments during the autumn of 1939.

to that on the lower; this is an additional advantage for the present assimilation experiments as has been mentioned in the previous paper (Heath, 1939).

The leaves used for experiments were in all cases fully expanded and of more than 4 in. diameter. At the same time leaves showing the slightest symptoms of senescence or insect punctures were avoided. In the case of Begonia the youngest fully expanded leaf on a shoot was always selected for experiment. Such leaves measured not less than 6 in. × 4 in.

The author is indebted to Mr. Robinson and his staff at the Chelsea Physic Garden for the skill and care with which the plants have been propagated and grown.

LEAF STRUCTURE

Since use will be made of various dimensions of the leaf and stomata in this and subsequent papers a brief account of the leaf structure[1] in the two species may be found useful for reference.

In the strain of Pelargonium used the leaf is mesophytic in type, about 180μ thick with a well-developed palisade layer about 36μ thick. In this layer most of the cells apparently touch their neighbours throughout their length so that the intercellular spaces run almost exclusively at right angles to the leaf surface. Any lateral movement of gas in the leaf, whether by diffusion or viscous flow, must therefore be almost entirely confined to the spongy mesophyll tissue, which including the 'connecting cells' at the base of the palisade is about 100μ thick. The intercellular spaces in the spongy mesophyll account for about 30 per cent. of the area as seen in transverse section. This figure is a mean value from camera lucida drawings of sections 2μ thick of material fixed in Navashin's fluid. The leaf is homobaric and there are considerable intercellular spaces in the mesophyll underlying the very small veins, but it is probable that the main veins provide an almost complete barrier to gas movement either by viscous or diffusive flow. In a large leaf there are spaces up to about $2\cdot5$ cm. wide between main veins where a small porometer cup may be affixed. The leaf chambers (Heath, 1939) cross the main veins more or less orthogonally and were in fact specially designed for the Pelargonium leaf. Both the upper and lower epidermis is hairy, especially the lower which also has far more stomata than the upper; the variation in stomatal numbers is discussed below (p. 478). The structure of the lower stomata is shown in Fig. 1 in which the average dimensions have been inserted. The full depth of the stomatal pore is about 12μ, but owing to the more or less funnel-shaped entrances to the pore the depth of the narrow part is only about 5μ in the centre of the elliptical opening. The long and short axes of the almost elliptical aperture are least in the midplane of the epidermis, and in this position their values vary from 10 to 20μ for the long axis and from 0 to 10μ for the short axis. These values for the two axes are

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[1] The author is indebted to Mr. P. S. Nutman, who very kindly embedded, cut, stained, and mounted the material used in obtaining these data.

obtained from measurements on strips of epidermis taken by Lloyd's method and their validity will be discussed in Part 3. On the outside of the stoma is a very delicate rim of cuticle which somewhat constricts the aperture at this point and appears in section as a pair of 'horns' (Fig. 1). The upper stomata are similar, but tend to be slightly larger.

Begonia sanguineum is also very suitable for the leaf chambers, which are
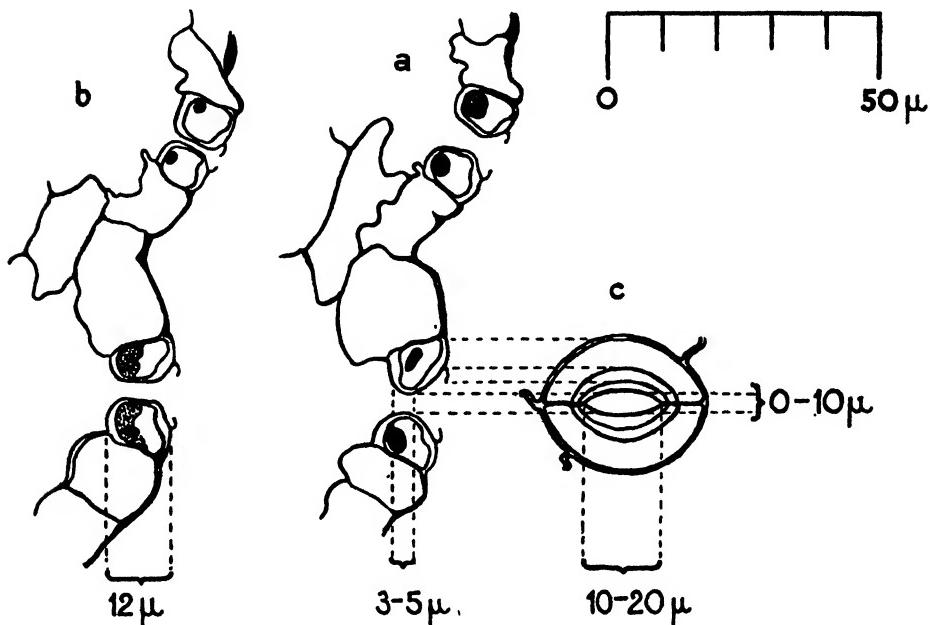
FIGS. 1 *a–c*. Lower stomata of *Pelargonium zonale*. The dimensions refer to average values. Fig. 1 *a*. Camera lucida drawing of transverse section through two open stomata, near centre of aperture. Fig. 1 *b*. Similar drawing near one end of the elliptical aperture. Fig. 1 *c*. Diagram of surface view of one of the stomata.

attached to the wider side of the asymmetrical leaf. Since the leaf is glabrous and very smooth it is easy to avoid air leaks into the chambers. Under each epidermis of the leaf is a thick layer of large-celled 'water-storage' tissue. Between these two layers is a palisade about $36\,\mu$ in thickness and a layer of spongy mesophyll of about $50\,\mu$ (Fig. 2). Owing to the tapering form of the palisade cells, the spaces in the lower part of this tissue must be available for lateral flow of gas. Stomata, which are present in the lower epidermis only, are grouped together in circular patches of four to ten each (Fig. 3). Abutting on each stomatal patch is a cylindrical tunnel passing through the water storage tissue to the spongy mesophyll (Fig. 2). Gaseous diffusion has therefore to traverse these tunnels which are about $180\,\mu$ long and $200\,\mu$ wide; and in the case of porometer experiments air or gas has to pass down one set of tunnels, along through the mesophyll and up a second set. Owing to the great width of the tunnels their resistance is likely to be low, but since the spongy

mesophyll layer is narrow and small veins and cystoliths appear to be numerous the leaf's total internal resistance both to diffusion and viscous flow
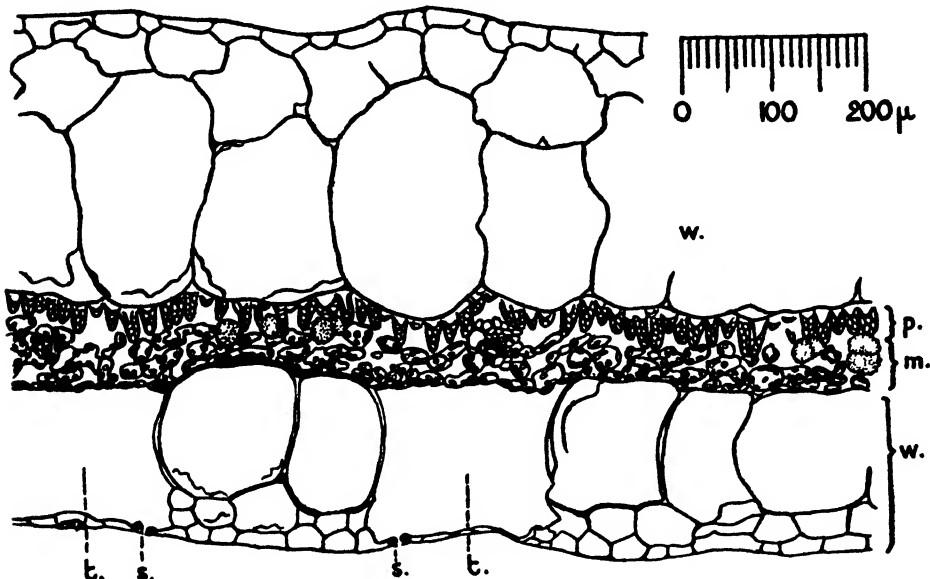
FIG. 2. Transverse section of leaf of *Begonia sanguineum*. *w*, water storage tissue. *p*, palisade parenchyma. *m*, spongy mesophyll. *s*, stoma. *t*, cylindrical tunnel abutting on stomatal group.
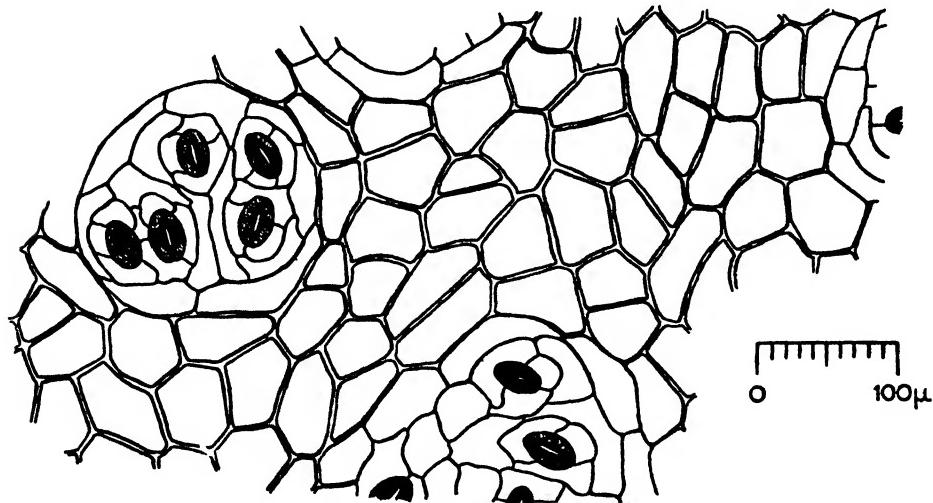
FIG. 3. Lower epidermis of leaf of *B. sanguineum*, showing groups of stomata.

may be expected to be higher than in Pelargonium. The stomata in any one patch are about 30–80 μ apart and the long axis of the elliptical opening varies between about 12 and 18 μ. No measurements of the short axis have been

made. The total depth of the pore is about 10 μ, but as in the case of Pelargonium the depth of the narrow part is less and approximates to 4 μ. The Begonia stoma also has a cuticular rim giving the effect of 'horns' in transverse section.

<div align="center">THE RESISTANCE POROMETER</div>

<div align="center">1. *Theoretical Considerations*</div>

The resistance porometer has been fully described by its originators (Gregory and Pearse, 1934), but a diagram of the form used in the present investigations (Fig. 4) will clarify the following discussion. Air is drawn through the leaf R_2 into a porometer cup by means of a constant pressure aspirator P, one of two alternative standard capillary resistances at R_1 being placed in series with the leaf resistance R_2, i.e. between the cup and aspirator. A water manometer p_2 measures the pressure drop (P_1-P_2) across the leaf R_2 and a second manometer p_1 measures the total pressure drop (P_1-P_3) across R_2+R_1.[1] By difference the pressure drop across the series resistance R_1 is P_2-P_3. It will be noted that the actual measurements made are in terms of pressure differences. These may be used for calculating either the resistance or the conductance of the portion of the leaf through which air flows.

(a) *The resistance formula.* The usual formula, as given by Gregory and Pearse, assumes that the volume V of air flowing through a constant resistance in unit time is proportional to the pressure difference. Thus

$$V = \frac{(P_1-P_2)}{R_2}k_2 = \frac{(P_1-P_3)}{R_1+R_2}k_3 = \frac{(P_2-P_3)}{R_1}k_1, \qquad (i)$$

where V is measured at constant pressure. This is made clear from the geometry of the diagram (Fig. 5). Hence assuming that k has a constant value

$$R_2 = \frac{R_1(P_1-P_2)}{(P_1-P_3)-(P_1-P_2)} = \frac{R_1(P_1-P_2)}{(P_2-P_3)}. \qquad (ii)$$

Actually the general expression for gaseous flow through a capillary involves the difference of the squares of the pressures at each end, but except when the pressure drop is large the error involved in the simpler assumption is negligible. The formula found for R_2, assuming that flow through both the leaf and the series resistance is proportional to the difference of the squares of the pressures, is

$$R_2 = \frac{R_1(P_1-P_2)(P_1+P_2)}{(P_2-P_3)(P_2+P_3)}. \qquad (iii)$$

Since (P_1-P_3) never exceeds 13·6 cm. of water in the present investigations the error in taking (P_1+P_2) as equal to (P_2+P_3) does not amount to more than

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[1] As mentioned in the previous paper, the necessity for reading p_1 lies in the fact that at high rates of flow the suction produced by the aspirator falls appreciably owing to the resistance to outflow of water.

To aspirator P

P_1

R_1

R_2

P_2
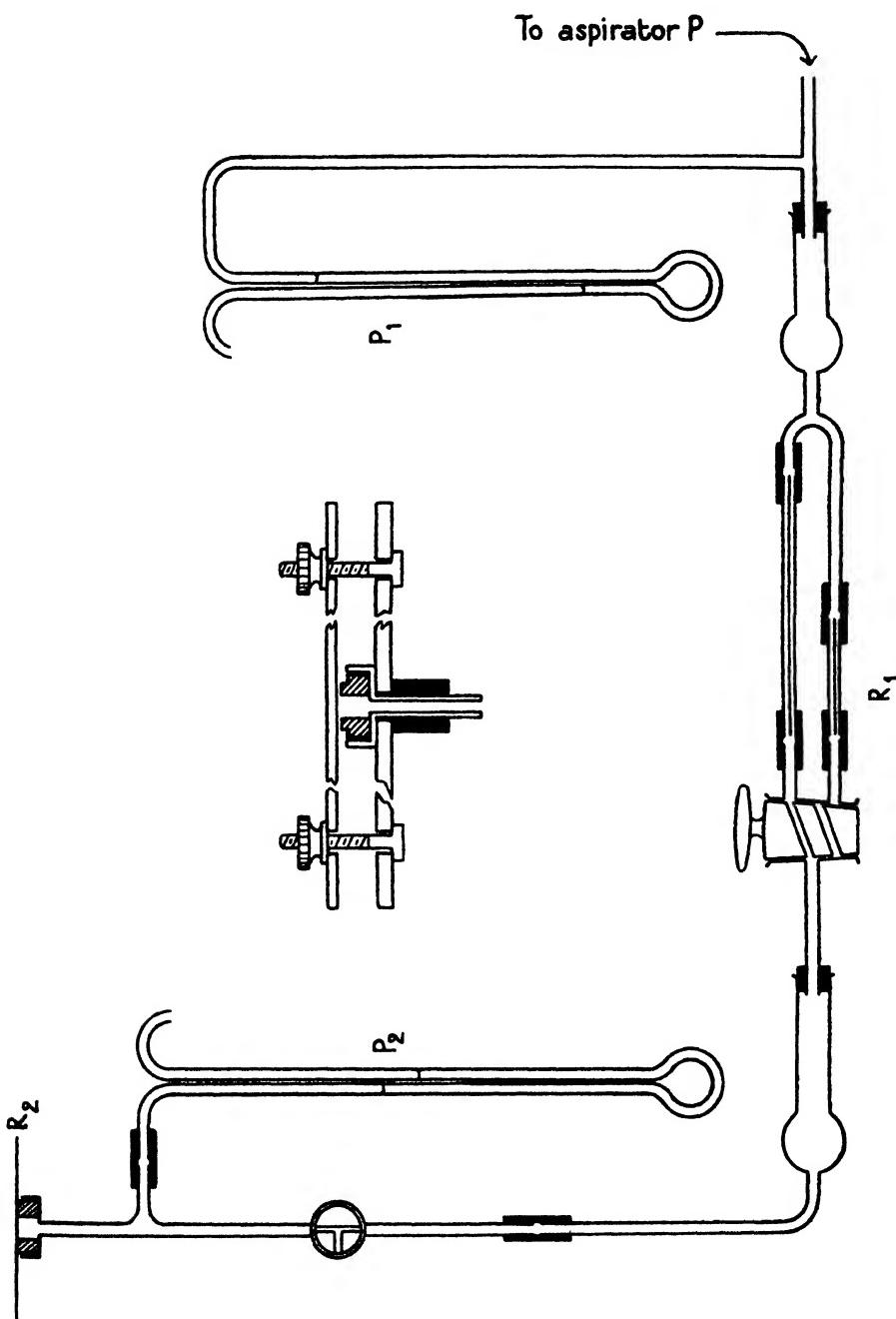
Fig. 4. Diagram of the resistance porometer employed. The enlarged inset shows the method of supporting the small circular porometer cup against leaf. For explanation of figure see text.

about 0·7 per cent. Equation (iii) then reduces to equation (ii). If very large heads are used in the constant pressure aspirator, especially when extreme accuracy is required, it may be better to use the more cumbersome equation (iii), necessitating the use of the absolute pressures.

(b) *The unit of resistance.* It has been assumed above that k has the same
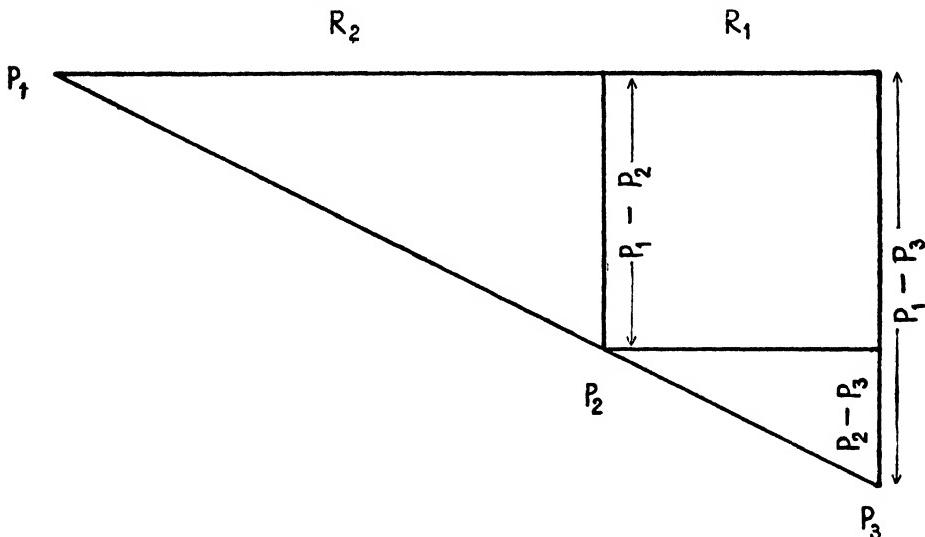
FIG. 5. Diagram illustrating the pressure relations of the resistance porometer.

value for the leaf as for the capillary tube R_1. In the present work this follows from the definition of resistance used, which may be stated thus: the resistance R of a portion of leaf or a capillary tube is said to be the dimensional value l/r^4 cm.$^{-3}$ of an equivalent long capillary tube of circular cross section, having a length l and radius r, which would pass the same flow V of the same fluid for the same pressure drop, i.e.

$$R = \frac{l}{r^4} = \frac{(P_1-P_2)}{V} \frac{\pi}{8\eta}. \tag{iv}$$

R is thus a dimensional constant obtained by assuming only that flow is proportional to pressure drop, and by definition k_1, k_2, and k_3 in equation (i) have all been made equal to $\pi/8\eta$, η being the coefficient of viscosity.

The leaf resistance R_2 is thus found in terms of the capillary resistance R_1 defined as above.

For convenience, arbitrary capillary units are used, except where otherwise stated, such that one unit = 3.77×10^8 cm.$^{-3}$ (Heath, 1939); this being approximately the same as the unit used by Gregory and Pearse (1934).

The values of R_2 are dimensional, but it does not necessarily follow that the term r^4/l in Poiseuille's formula, or the equivalent expression $2a^3b^3/(a^2+b^2)l$ for an elliptical capillary tube of semi-axes a and b, is correct in terms of the

actual stomatal dimensions, although it is satisfactory as a value for the con-
ductance of a capillary tube equivalent to the leaf.

(c) *The validity of the resistance determinations.* The assumption that flow
through both the leaf R_2 and the series resistance R_1 is proportional to the
pressure drops across them, or alternatively to the differences of the squares of
the pressures, is vital to the use of the resistance porometer; for if it is appre-
ciably incorrect equations (ii) or (iii) may no longer hold and R_2 may be
wrongly estimated. Proportionality between flow and pressure drop, indicat-
ing that the former is predominantly viscous in character, may be tested
by direct experiment. Such tests were carried out (Heath, 1939, p. 490)
for the capillary resistances A and B used at R_1 in these investigations, and
flow was found to be proportional to pressure difference up to rates greatly in
excess of those used in experiments with leaves. Similar tests may be carried
out for flow through a leaf attached to the resistance porometer by taking
alternate readings with the two standard resistances. Since one of these (A)
has approximately a quarter of the resistance of the other (B), it gives a much
larger pressure drop $(P_1 - P_2)$ across the leaf. If the same value for R_2 is found
with A instead of B at R_1 the rate of flow has increased proportionately with
$(P_1 - P_2)$. The results of such experimental tests (see section 2 (c) below) give
no evidence of non-viscous flow through the leaf. Unfortunately these experi-
ments could not be satisfactorily carried out at the smallest stomatal apertures,
although they cover the major part of the range of leaf resistance usually en-
countered. With very high leaf resistances the use of the lower resistance A
at R_1 gives very poor sensitivity owing to the great difference between the
values of R_1 and R_2 (see p. 467 below). At the highest leaf resistances,
although the narrowness of the stomatal apertures increases the likelihood of
turbulent flow, the very greatly reduced rate of flow acts in the opposite
direction. Actually Reynold's number is estimated as of the order of unity.[1]
It may be noted that Maskell (1928) working with cherry laurel found flow to
be proportional to pressure drop with a Darwin type porometer.

It has been shown that from resistance porometer readings valid estimates
of leaf resistance may be made on the apparently justifiable assumption of
proportionality between flow and pressure difference. It is of course equally
legitimate to use the resistance porometer readings for calculating the conduc-
tance $1/R_2$, of the leaf. In either case the measure obtained is independent of
the pressure drop employed and is in this respect preferable to measurements
of flow as obtained with the Darwin and Pertz (1911) or the Knight (1915)
porometer in that it more nearly approaches to a measure characteristic of the
leaf dimensions only. Values obtained with a flow type of porometer, if cali-
brated in actual rates of flow, may however be used to calculate the resistance
or conductance from equation (iv).

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[1] Reynold's number $= v\,dp/\eta$, where v = velocity of flow; d = diameter of tube; ρ =
density of fluid; η = viscosity of fluid. The critical value above which turbulent flow may be
expected to appear is approximately 2,000.

Over the range of leaf resistance covered by the experiments described in section 2 (*c*) and mentioned above the proportionality found between flow and pressure drop disposes of the criticism (cf. Darwin, 1916; Knight, 1916; and Stålfelt, 1929) that the flow of air through the stomata may force them open to an appreciable extent.

(*d*) *Size of cup and sensitivity.* The use of a small cup for calibrations is made necessary by practical considerations, such as the need for many positions on the same leaf for calibration in terms of stomatal aperture, and the desirability of keeping the area, and hence the diffusion rate, reasonably small for calibration in terms of diffusive resistance.[1] For the actual assimilation experiments a large porometer cup, such as the leaf chamber *LI*, Heath (1939), is advantageous because, with the relatively low values of R_2 that it gives, equilibrium is more rapidly attained. Furthermore, the manometer reading (p_2) oscillates more rapidly and with less amplitude with the bubbling in the aspirator when R_2 is low, and therefore the mean reading may be obtained with greater speed and accuracy. The actual shape of *LI* was of course dictated by the shape of the Pelargonium leaf and the necessity for avoiding the thicker parts of the veins, yet having a sufficiently large area for the assimilation measurements.

In relation to sensitivity it seems to be a matter of indifference whether a large or small cup is used, provided that a suitable resistance is chosen for R_1. Gregory and Pearse (1934) showed that for given values of $(P_1 - P_3)$ and R_1 the sensitivity of the resistance porometer, i.e. the change in $(P_1 - P_2)$ for unit change of R_2, was greatest when R_2 was minimal, and this would appear to favour a large cup. On the other hand, maximum sensitivity in terms of conductance $1/R_2$, i.e. the greatest change in $(P_1 - P_2)$ for unit change of $1/R_2$, is obtained when R_2 is maximal which seems to favour a small cup. If use is made of $\log R_2$ or $\log 1/R_2$, the change in $(P_1 - P_2)$ for unit change of $\log R_2$ is the same as that for unit change of $\log 1/R_2$. Moreover this sensitivity would be constant for all values of R_2, if R_1 could be maintained almost equal to R_2. Gregory and Pearse (1934) give the equation for sensitivity in terms of R_2, which in our notation is:

$$\frac{d(P_1 - P_2)}{dR_2} = \frac{(P_1 - P_3)R_1}{(R_1 + R_2)^2},$$

where R_1 and $(P_1 - P_3)$ are maintained constant. From this it may be shown that the sensitivity in terms of $\log_e R_2$ is:

$$\frac{d(P_1 - P_2)}{d\log_e R_2} = \frac{(P_1 - P_3)R_1}{(R_1 + R_2)^2} R_2.$$

Hence, if R_1 is made equal to R_2,

$$\frac{d(P_1 - P_2)}{d\log_e R_2} = \frac{(P_1 - P_3)}{4}.$$

[1] With Begonia, in which both the flow and diffusive resistances are much higher than in Pelargonium, a rectangular cup 8 × 1 cm. is used for calibrations instead of a small one.

If, therefore R_1 can be maintained approximately equal to R_2, the sensitivity in terms of $\log_e R_2$ (or $\log_e 1/R_2$) is constant at a value equal to $1/4$ of the pressure drop across the whole system. The corresponding sensitivity in terms of $\log_{10} R_2$ is of course $2 \cdot 303(P_1 - P_3)/4$.

When $R_1 = R_2$, sensitivity is maximal not only in terms of R_2, as was shown by Gregory and Pearse, but also in terms of $\log R_2$ or $\log 1/R_2$. This may be shown by differentiating (P_1-P_2) a second time with respect to $\log_e R_2$:

$$\frac{d^2(P_1-P_2)}{d(\log_e R_2)^2} = \frac{(P_1-P_3)R_1 R_2}{(R_1+R_2)^2}\left\{1 - \frac{2R_2}{(R_1+R_2)}\right\},$$

where R_1 and (P_1-P_3) are maintained constant. Equating this to zero gives maximum sensitivity when $R_1 = R_2$.

If R_1 is of the same order as R_2, the change in sensitivity in terms of $\log R_2$ or $\log 1/R_2$ is found to be small over a large range of R_2, e.g. if (P_1-P_3) is maintained constant at 10 units and R_2 is increased or decreased five-fold above or below R_1, giving a total range of twenty-five times, the sensitivity in terms of $\log_e R_2$ falls off symmetrically on either side of the maximum value of $2 \cdot 50$ to $1 \cdot 39$ at the extremes of the 25-fold range of R_2. On the other hand, for a 25-fold increase in R_2 the sensitivity in terms of R_2 decreases $7 \cdot 4$ times when, in order to secure throughout the best sensitivity, R_1 is made equal to the mean of the extreme values of R_2. When $R_1 = R_2$, the sensitivity in terms of R_2 is $(P_1-P_3)/4R$ and thus depends on the value of R_2, unlike that in terms of $\log R_2$ given above. This relatively small variation in sensitivity in terms of logarithmic values makes it desirable from a statistical point of view to use such values whenever possible in order to maintain the errors as constant as may be, since the error of reading the manometer p_2 is approximately the same throughout the whole range.[1] It is also of course desirable to maintain R_1 as similar to R_2 as is practicable, and a glance at the level of manometer p_2 will show when this is achieved, since $(P_1-P_2) = \frac{1}{2}(P_1-P_3)$ when $R_1 = R_2$.

The above considerations with regard to sensitivity of course apply to the total resistance R_2 or conductance $1/R_2$ as measured. When calculated values of $1/s_1$ or s_1 (see next section) are used, the results given above will be modified especially at large stomatal apertures where the correction required for the effect of the resistance of the intercellular spaces and the stomata outside the cup is great.

(e) *The relation between stomatal resistance and total resistance measured with a circular porometer cup.* All porometer methods are open to the criticism that the readings are affected not only by the resistance of the stomata within the cup but also by the resistance of the intercellular spaces and the stomata outside the cup. The combined resistance of the stomata outside the cup would be negligible, since their number is usually large compared with the number

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[1] Actually the manometer readings are slightly more accurate at lower values of (P_1-P_2) owing to the smaller and more rapid oscillation mentioned earlier.

within the cup, were it not for the effect of resistance to flow in the inter-cellular spaces. The existence of the latter, which for convenience may be termed 'mesophyll resistance' since nearly all lateral flow must take place through the spaces in the spongy mesophyll, adds greatly to the complexity of the problem.

Stålfelt (1929) considered that the presence of the mesophyll resistance and the existence of other sources of error rendered impossible the finding of a relation between air flow and stomatal aperture. Darwin (1916) concluded that the stomata within the cup provided the limiting control of air flow since cutting the leaf near the cup did not seem to increase the rate. He appears to have overlooked the possibility of infiltration of the intercellular spaces by sap from the injured cells and of 'shock' closure of the stomata within the cup.

Knight (1916) measured the reduction of flow caused by blocking the stomata over a known area surrounding the porometer cup, using either vase-line or his ingenious double cup. In this way he estimated the effect of the mesophyll resistance for an extra length of path through the leaf. He con-cluded that this resistance was considerable and might markedly affect poro-meter readings, and he considered that since it was approximately constant its relative importance increased as the stomata opened. Newton (1936) drew attention to the following important consideration: '. . . as the stomata open the flow through those remote from the cup will be less, and hence less meso-phyll will be included in the path of flow.' He made the first attempt known to the author to partition the various measured total resistances R into com-ponent resistances attributable to the stomata within the cup R_l, to those immediately above it R_u, and to the intercellular spaces plus other stomata outside the cup R_m. For this purpose he made use of an electrical analogy, devised by Mr. Baggally, in which the leaf was considered as a system of three plates pressed together. The two outer plates representing upper and lower epidermis respectively conducted only at right angles to their plane surface with conductances in the ratio of the numbers of stomata per unit area. The middle sheet representing the other leaf tissues ('mesophyll') was assumed to be without resistance at right angles to the leaf surface, being very thin compared with the radius of the leaf, but to have a superficial resistivity m. Current was supposed to flow from a circular central electrode representing the porometer cup on the lower surface of the leaf, through the stomata inside the cup, along the mesophyll, and to leak away through all the remaining stomata to an earthed conducting fluid bathing the leaf outside the cup. The system was split into two parts, that opposite the cup where the current passed directly through the leaf and that beyond the cup where the current passed along the mesophyll and leaked away through the remaining stomata.

Newton estimated m, the superficial resistivity of the mesophyll, by means of double cup experiments similar to those of Knight (1916), but he seems to have overlooked the fact that this should have been done at the widest possible stomatal apertures so that with the outer cup open the minimum of air would be

flowing through the mesophyll.[1] Actually all his three double-cup experiments were carried out at medium stomatal apertures and all gave almost the same resistance for the extra area of mesophyll. His value of m must therefore have been an underestimate. He assumed a ratio of lower to upper stomata of 4 to 1, and for various assumed values of stomatal conductance s (where $s = 4s/5 + s/5$) he calculated R_l, R_u, and R_m. Hence he obtained the total resistance R from

$$R = R_l + \frac{R_u R_m}{R_u + R_m}.$$

He found that over most of the very great range of R that he explored experimentally R_l accounted for almost the whole resistance, but at maximal stomatal openings $R_u R_m / (R_u + R_m)$ became important.

He also calculated from his electrical analogy the percentages of the total air current which passed straight through the leaf or across rings of stated radius round the cup. Owing to relative changes in R and R_m the distribution of the air stream was found to differ greatly at large and at small stomatal openings. Thus for a cup of radius 0·5 cm. at the smallest apertures 0·21 per cent. passed straight through above the cup and 97 per cent., 85 per cent., and 36 per cent. across rings of radius 1, 2, and 4 cm. respectively. At the widest aperture on the other hand 14 per cent. of the air passed straight through and 15 per cent., 0·38 per cent., and 0·0001 per cent. across the above-mentioned rings.

The treatment used by Newton appears open to two main criticisms. The first is that no account is taken of the resistance to lateral flow in the mesophyll above the cup, since he assumes in the calculations that current flows directly through the leaf to the upper stomata above the cup or else laterally from the edges of the cup. Actually there would also be some lateral flow above the cup. The second criticism is that no account is taken of the effect of the width of the washer attaching the porometer cup to the leaf. To complete the analogy this should be represented by a ring-shaped insulator surrounding the central electrode. A third criticism is to be levelled at Newton's apparent assumption that for the purposes of the analogy the radius of the Pelargonium leaf might be taken as 10·0 cm. (Newton, 1936, p. 23). For a circular porometer cup of 0·5 cm. radius the mean radius of surrounding leaf tissue unobstructed by main veins would be nearer 2·0 cm., and certainly not more than 3·0 cm. even for the largest leaf. This might be expected to modify considerably the calculated values of R_m and the theoretical distribution of flow in the different parts of the leaf.

Dr. H. L. Penman has very kindly made a new mathematical investigation of the problems of distribution of air flow in a Pelargonium leaf with a porometer cup attached. The problem is treated on lines somewhat similar to that of the electrical analogy used by Newton, but allowance is made for the

[1] Newton calculated $m = \dfrac{2\pi(R_2 - R_3)}{\log_e a_3/a_2}$ with no further correction (cf. Appendix, p. 495).

effects of lateral flow above the cup and of the width of the washer. In this investigation the following assumptions have been made:

1. That the rate of air flow varies directly as the pressure difference. This seems justifiable since the pressure differences are small and lack of turbulence has been shown experimentally over a considerable range of stomatal opening (see section 2 (c) below).

2. That flow at the boundary, a distance b from the centre of the cup, is zero. The value chosen for b is the approximate mean distance of the edge of the leaf and of main veins.

3. The conductances s_2 and s_1 of the upper and lower epidermis respectively are assumed to be in the ratio of the numbers of stomata. This implies negligible interference between the stream lines flowing through neighbouring stomata and also that the mean stomatal apertures are at all times the same on both surfaces. Newton (1936) found that the stomata on the two surfaces opened and closed synchronously. The upper stomata have a tendency to be somewhat larger than the lower, when examined by Lloyd's method, but against this may perhaps be offset the resistance to flow in the spaces of the palisade parenchyma (see 4 below, but also section 2 (d) and Discussion).

4. The intercellular space resistance is assumed to be negligible in a direction at right angles to the plane of the leaf. This seems reasonable in view of the shortness of the path and the fact that intercellular spaces predominantly run in this direction. Such resistance as occurs in the palisade tissue will be additional to that of the upper stomata, and hence at large apertures there may be a tendency for s_2/s_1 to fall (but see section 2 (d) and Discussion).

5. Since it is assumed that the mesophyll has a resistance to flow only in directions parallel to the leaf surface (assumption 4) its 'specific resistance' m may be termed a surface resistivity. The value of m is assumed to be constant which implies no change in volume or shape of the intercellular spaces with stomatal movement. This seems likely to be a reasonable approximation as long as the leaf is not allowed to wilt,[1] and some experimental evidence for the constancy of m has been obtained (see section 2 (d) below).

The mathematical argument is presented by Dr. Penman in the Appendix. Briefly, it may be stated here that the system is considered in three parts: the area of leaf of radius a_1 covered by the circular cup, the area covered by the washer of width a_2-a_1, and the remainder of the leaf up to the boundary of radius b. In this outer part of the leaf air flows into an elementary annulus from above, below and the periphery and out of it towards the centre; above the washer air flows in from above and the periphery only and again leaves the annulus towards the centre; while over the cup air flows into such an

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[1] Pearse (1935) found that the leaf of Pelargonium wilted when the percentage water content of a fully turgid leaf fell by less than 2. The shrinkage resulting from this would have an entirely negligible effect on diffusive resistance and the effect even on flow resistance would not be large.

annulus from above and the periphery, and leaves it towards the centre and towards the cup. At the centre of the cup there is no lateral flow. From a theoretical consideration of these effects a series of equations has been obtained enabling $1/R_2$, the total conductance, to be calculated from the following physical characteristics of the system:

a_1 the radius to the inside of the washer,
a_2 the radius to the outside of the washer,
b the radius to the boundary,
m the surface resistivity of the mesophyll,
s_1 the conductance of the lower epidermis,
s_2 the conductance of the upper epidermis,

and from derived functions β_1 and β_2 which equal $\sqrt{\{m(s_1+s_2)\}}$ and $\sqrt{(ms_2)}$ respectively. The only unknowns are s_1 and s_2, which have a known ratio α, and m which can be evaluated by experiment. Modified equations for a hypostomatous leaf ($s_2 = 0$) have also been derived. By the use of these equations, values of m/R_2 can be calculated for various assumed values of β_1, and in this way a series of curves relating log m/R_2 to log ms_1 may be constructed for different values of α (the ratio s_2/s_1). From this known ratio and the experimentally determined value of m/R_2 the value of log ms_1 may be read off from the curves, either directly or by interpolation.[1] The calculation enabling the curves to be plotted proceeds by a series of steps using in turn the equations (4), (5) and (6 a) given in the Appendix. For the hypostomatous leaf ($\alpha = 0$), the term in the square bracket on the left-hand side of equation (4) [M of equation (7)] and equation (7) only are needed. A sample calculation for an amphistomatous leaf ($\alpha > 0$) will now be presented to demonstrate the method of procedure:

Let $a_1 = 0\cdot45$; $a_2 = 0\cdot70$; $b = 2\cdot0$; $\alpha = 0\cdot04$, and take a value of β_1 such that $\beta_1 a_1 = 0\cdot20$.

With these values the term in the square bracket on the left-hand side of equation (4) of the Appendix (M of equation 7) is found to be $-1\cdot073$.

Hence $\quad \dfrac{\beta_2}{\beta_1}M = \sqrt{\left(\dfrac{s_2}{s_1+s_2}\right)}M = \sqrt{\left(\dfrac{\alpha}{1+\alpha}\right)}M = -0\cdot210$

and $\qquad\qquad\qquad F/G = +0\cdot487.$

Hence right-hand side of (5) $\quad = 0\cdot152 = N.$

Then $\qquad \dfrac{m}{R_2} = \dfrac{2\pi\beta_1 a_1}{(1+\alpha)^2}\left[\dfrac{\alpha}{2}\beta_1 a_1 - \dfrac{1}{\sqrt{\left(\dfrac{1+\alpha}{\alpha}\right)}N - \dfrac{I_0(\beta_1 a_1)}{I_1(\beta_1 a_1)}}\right].$ (6 a)

$\qquad\qquad\qquad = 0\cdot111.$

Also, $\qquad ms_1 = \dfrac{\beta_1^2}{1+\alpha} = 0\cdot190.$

[1] Note that R_2 is the total resistance found with the size of cup used and s_1 is the stomatal conductance per unit area of lower epidermis.

A set of three typical curves is shown in Fig. 6. They relate to a circular cup of radius 0·45 cm. having a washer 0·40 cm. wide, with an assumed value for the distance of the boundary b of 2·0 cm. The three curves are for three different ratios (α) of upper to lower stomatal numbers, namely 0·00, 0·04, and 0·10.

In order to use such curves for calculations m must be known. In the present
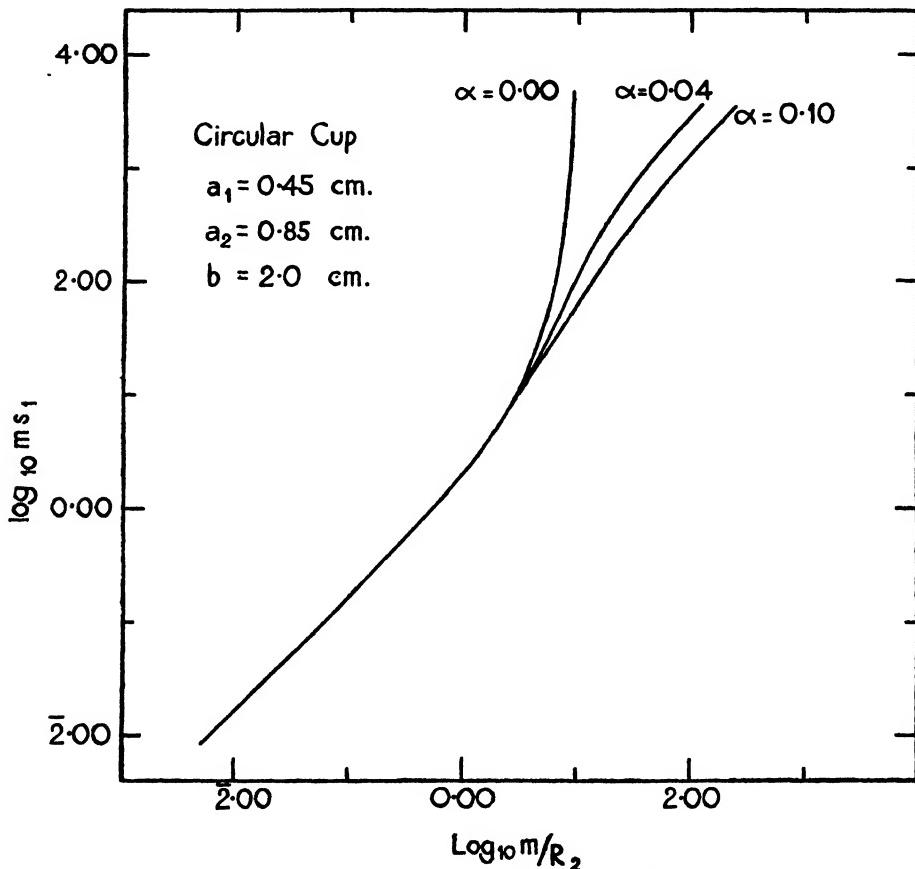
FIG. 6. Curves illustrating theoretical relationship between total resistance R_1 and stomatal conductance per unit area s_1.

investigations the leaf chambers (Heath, 1939) have been used for double-cup experiments (see section 2 (*d*) below) and m has been estimated by the method of successive approximation given in the second part of the Appendix (p. 497). The value of 4·85 units found for Pelargonium is derived from experiments with only two leaves, while that for Begonia (16·6) is based on a single leaf. Obviously if a single value of m is to be used for all leaves of the same strain it should be based on much wider experimentation. It would also have been preferable to carry out the double-cup experiments with a circular double cup

similar to Knight's. As mentioned in the Appendix, the theory of flow distribution for a circular cup involves fewer approximations than that for an elongated cup, especially in the case of the outer part of a double cup when a_2 is large. If the inner circular cup were placed symmetrically across a major vein a reasonably large area of leaf unrestricted by veins would be obtained beyond the outer cup. (See Appendix, p. 495, where the method of calculating m from the results of experiments with a circular double cup is described.) It is hoped to carry out such further experimentation at a future date. Meanwhile the approximate values of m obtained will serve to show the types of results that may be expected. It may be mentioned that the two double-cup experiments carried out in May and December respectively with Pelargonium leaves of very different size gave very consistent estimates of m.

Using the above-mentioned values of m the extreme range of log ms_1 in the author's experiments is from $\bar{2}\cdot4$ to $3\cdot3$, this being for Pelargonium. These figures show that practically the whole of the range plotted in Fig. 6 may be needed, although of course that usually encountered is much smaller, say $0\cdot0$ to $2\cdot5$ for Pelargonium and $\bar{1}\cdot5$ to $1\cdot0$ for Begonia. Dr. Penman has pointed out (Appendix, p. 499) that the value of m becomes extremely critical when the slope of the curve exceeds 2, and it is worthy of note that if α is not less than $0\cdot04$ the slope scarcely exceeds 2 on any part of the curves for the cups used, whether circular or elongated. For a hypostomatous leaf such as Begonia ($\alpha = 0$) the slope of the curve does not reach 2 until log ms_1 reaches a value of at least $0\cdot8$.

It may be useful to tabulate for Pelargonium some typical calculated values of R_2 and $1/s$ falling within the observed range of log ms_1, in order to demonstrate the importance of correcting R_2 for the effects of the mesophyll and outer stomatal resistances. Since $1/s$ represents the stomatal resistance within the cup and R_2 the total resistance the difference between these values is a measure of mesophyll plus outer stomatal resistance. (See Table I.)

TABLE I

Pelargonium

Values of Total Resistance, R_2, with a circular Cup of $0\cdot636$ cm.^{2} area corresponding to various stomatal Resistances ($1/s$ per $0\cdot636$ cm.^{2} of lower epidermis)

$a_1 = 0\cdot45$; $a_2 = 0\cdot85$; $b = 2\cdot0$; $m = 4\cdot85$

log ms_1	$1/s$	R_2		
		$\alpha = 0\cdot0$	$\alpha = 0\cdot04$	$\alpha = 0\cdot10$
$\bar{2}\cdot4$	307	331	331	331
$\bar{1}\cdot0$	76·9	83·3	83·3	83·3
0·0	7·69	8·93	8·93	8·93
1·0	0·769	1·70	1·62	1·51
1·5	0·243	1·05	0·870	0·775
2·0	0·0769	0·775	0·513	0·380
2·5	0·0243	0·662	0·282	0·166
3·0	0·00769	0·588	0·123	0·0645
3·3	0·00386	0·562	0·0690	0·0347

For the two smallest values of log ms_1 tabulated, the difference between $1/s$ and R_2, amounting only to 7–7·5 per cent., is attributable almost entirely to the finite value of b, i.e. to the limited number of stomata outside the cup. This is shown by the fact that the ratio, 0·94, of the reciprocal of the area inside the cup to the sum of the reciprocals of the areas inside and outside is almost the same as the ratio of $1/s$ to R_2, namely 0·93. As the stomata open, the importance of b rapidly diminishes and hence even large errors in its value are relatively unimportant as long as $b^2 - a_2^2$ is large compared with a_1^2. On the other hand the effect of the upper stomata increases rapidly in importance with increasing stomatal conductance. This can be clearly seen both from the curves in Fig. 6 and the values in Table I. It is evident that for the dimensions under discussion and with a hypostomatous leaf ($\alpha = 0$), values of R_2 are a very poor measure of stomatal resistance when log ms_1 exceeds 2·0. This is because R_2 is tending to a constant value, 0·492, given by equation (10) of the Appendix and due entirely to the resistance of the mesophyll above the washer. Under these circumstances not only will the value of m be very critical, but any errors in measuring the width of the washer will have a disproportionately large effect on the value of $1/s$ obtained. It is evident that porometer determinations without correction for mesophyll resistance will lead to greater errors in the case of hypostomatous than amphistomatous leaves, as has been pointed out by Stålfelt (1929) and Newton (1936), though even in the latter case these errors may be considerable at wide or medium apertures unless α is very large.

The greater the value of α, the more nearly does the curve relating log ms_1 to log m/R_2 approach to a straight line and hence the smaller the errors in uncorrected resistances.

Some of these consequences of the theoretical treatment are illustrated, from actual experimental data, by Fig. 7 which shows time curves for logarithms of total resistance R_2 and stomatal resistance $1/s$ for the same cup area during stomatal opening and closure. Values of log ms_1 are also plotted to indicate the position on the theoretical curves of Fig. 6. The curves relate to two experiments, one (28.11.39) with a hypostomatous and the other (16.11.39.) with an amphistomatous Pelargonium leaf; in the latter the ratio of upper to lower stomata was 0·10. The dimensions of the cups were the same as in Table I. The curves clearly show (1) that at high resistances log $1/s$ differs by an almost constant amount from log R_2, this being almost entirely an effect of b, (2) that at low resistances with the hypostomatous leaf very small changes in log R_2 correspond to large changes in log $1/s$, whereas with the amphistomatous leaf R_2 provides a more sensitive measure of $1/s$ although here also the difference between log R_2 and log $1/s$ is considerable.

(*f*) *The relation between stomatal resistance and total resistance measured with an elongated porometer cup.* The Appendix also includes an approximate theory of distribution of air flow in a leaf with an elongated porometer cup, such as the leaf chamber *LI* (Heath, 1939) or the rectangular cup 8 × 1 cm.,

used for certain experiments with Begonia. Here it has been found necessary to make the simplifying assumptions that the cup is a rectangle of length l
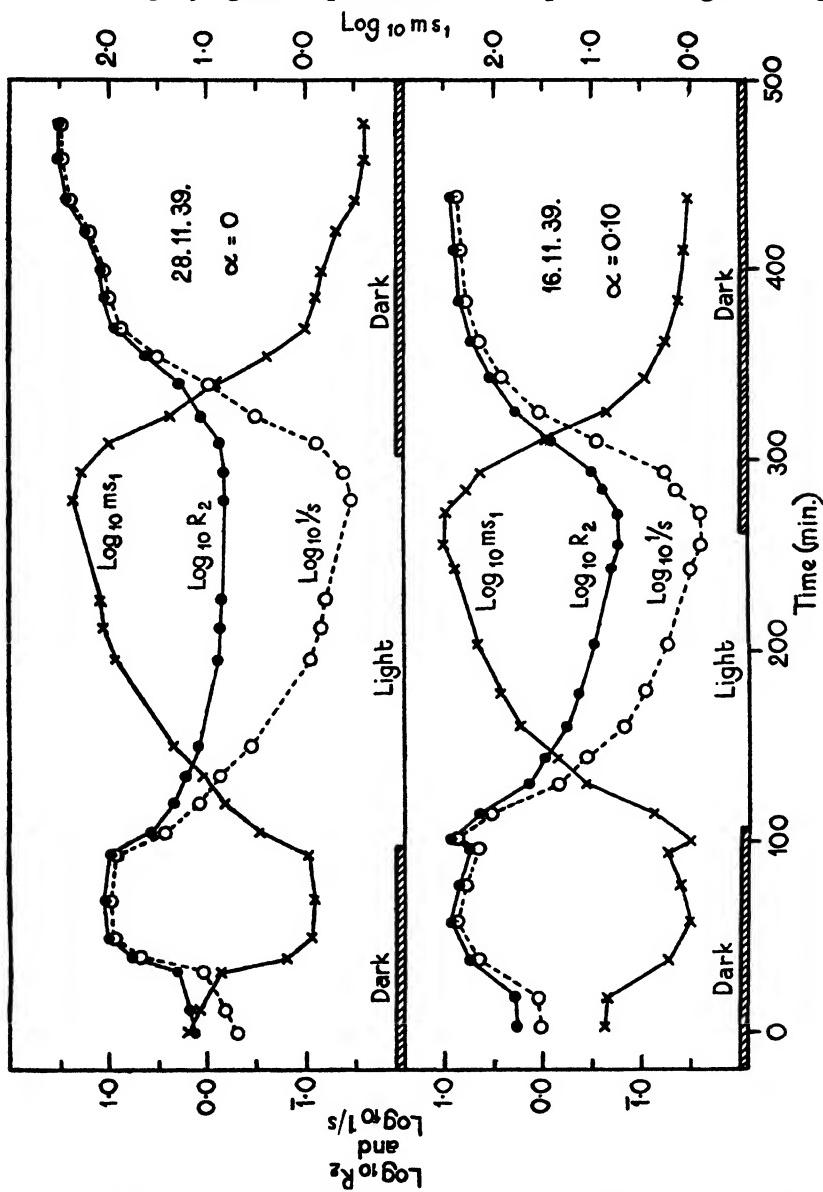
FIG. 7. Pelargonium. Variation in logarithms of total resistance R_2, stomatal resistance within the cup $1/s$, and ms_1 during stomatal opening and closure.

(equal to the area divided by the width $2a_1$) and that the effects of flow across the ends of the cup are negligible. The effects of the upper washers of the leaf chambers and of that surrounding the lower outer chamber LO have also been

neglected, while the other assumptions remain the same as previously stated for a circular cup. The value chosen for b is the approximate mean distance from the centre line of the cup to the edge of the leaf on one side and the main veins on the other. a_1 and a_2 are the distances from the centre line to the inside and outside respectively of the washer surrounding LI. From the equations (12) and (13) of the Appendix values of m/R_2 corresponding to assumed values of β_1 can be calculated. Curves relating $\log m/R_2$ to $\log ms_1$ can be plotted for different values of α. These curves resemble those for a circular cup and similar considerations apply to them. At small values of $\log ms_1$ b is more important than for a circular cup of the same a_1 and a_2 at the same value of b, but again the effect of b disappears rapidly as the stomata open. The neglect of the effect of the washer surrounding LO will also be of most importance at small stomatal apertures. For the dimensions used, the effect of the upper stomata is apparent in the separation of the curves at a lower value of $\log ms_1$ than for a circular cup.

Since the theory for the leaf chamber is much less precise than that for a circular cup, experimental tests of its validity are especially desirable. Three types of experiment which may give information as to the adequacy of the theory have been carried out:

First, the lower leaf chambers have been used as a double cup for the estimation of m with the upper chambers closed so as to simulate hypostomatous conditions. If, in such experiments, the value of m obtained by the method of successive approximation (Appendix, p. 497) is found to be nearly constant over a considerable range of stomatal conductance, this may be taken as evidence both for the constancy of m and for the correctness of the *shape* of the $\alpha = 0$ curve over the range studied. It should be pointed out that any error acting as a multiplying constant, e.g. the effective value of l being different from that actually used, will not affect the constancy but only the value of m found as above. Such an error will merely displace the curve relating $\log m/R_2$ to $\log ms_1$ without altering its shape.

Secondly, experiments have been carried out with the leaf chambers in which comparisons between the resistances R_h under hypostomatous and those R_a under amphistomatous conditions have been made by closing or opening the upper chambers. Hence curves relating $\log R_h/R_a$ to $\log m/R_a$ may be drawn for the appropriate values of α. Similar curves may be derived from the theoretical $\log m/R_2$ v. $\log ms_1$ curves by taking the differences $\log m/R_a$ minus $\log m/R_h$ (i.e. $\log R_h/R_a$) at given values of $\log ms_1$; these differences being plotted against $\log m/R_a$. The observed and theoretical curves should agree.

Both the above tests might be made for a circular cup also, given the necessary apparatus, and it is hoped to carry out the first of them at some future date.

Thirdly, comparisons have been made between stomatal conductance derived from the total resistance as measured with the leaf chamber LI on

one part of a leaf and that obtained at the same time with a circular cup on another part of the same leaf. If it is assumed that the theory for the circular cup is adequate and that the stomata open to the same extent in different parts of the leaf agreement between the values obtained over a wide range of stomatal conductance will provide evidence of the validity of the theory for an elongated cup.

As described in the previous paper, when the leaf chambers are used for assimilation experiments in which air is drawn *through* the leaf tissues it is necessary to trim off the parts of the leaf outside the outer chambers and grease the cut edges in order to prevent errors due to lateral diffusion of carbon dioxide. This reduces b to a very low value ($1 \cdot 1$ cm.), but although the effect of the resistance of the stomata outside the cup is thereby greatly increased there is the advantage that the value of b is known with some accuracy. Unfortunately, during an 'over' experiment which is to be followed by a 'through' experiment it is necessary to have the knife in position round the part of the outer chambers nearest the edge of the leaf. This restricts the area available on that side, for inflow of air, to the outer chamber ($b = 1 \cdot 1$), although on the petiole side of the chambers there is no such restriction ($b = 3 \cdot 0$ say). A mean value of b, generally $2 \cdot 0$, is therefore used as an approximation in such experiments. In order to test this approximation comparisons have been made of the results obtained with the leaf chamber with the knife in position and those obtained with a small cup on the same leaf.

2. *Experimental*

(*a*) *Apparatus.* Up to the summer of 1939 the resistance porometer apparatus used was that described in the previous paper, except that for some purposes the leaf chamber *LI* was replaced by a small porometer cup. This was a circular brass cup supported in an ebonite frame and holding a gelatine washer about $0 \cdot 25$ cm. wide and of about $0 \cdot 45$ cm. inside radius (a_1). The surface of the washer was greased as described for the leaf chambers in the previous paper and the leaf was pressed lightly against it by means of a glass or perspex plate held by two screws in the ebonite frame (Fig. 4). For the comparison of the resistance and diffusion porometers special cups were used which will be described in the appropriate context in Part 2 of this series. In these the internal radius a_1 of the gelatine washer was again $0 \cdot 45$ cm., but its width was $0 \cdot 40$ cm. After September 1939 porometer experiments were carried out in a field laboratory belonging to the Imperial College Laboratory at Rothamsted Experimental Station. The same capillary resistances A and B were used, and manometer p_2 was of precision bore tubing and read with a horizontal microscope as before. Manometer p_1, however, was of ordinary tubing, though selected for even bore, and it was therefore necessary to read both limbs.

It should be mentioned that tests showed the resistance to air flow of the taps and calcium chloride tubes of the apparatus to be negligible.

(b) *Areas and stomatal numbers.* After a porometer experiment the cup is removed and strips of epidermis are taken within and above the cup area, using Lloyd's method, for counts of stomata on the two surfaces. It will be shown in Part 3 of this series that this method gives valid estimates of number of stomata per unit area. The area of leaf covered by the cup is measured. In the case of the small circular cup this is generally effected by measuring several diameters with an accurate scale under a dissecting microscope. For the leaf chamber LI the outline of the chamber as shown by the grease is traced through to a sheet of paper by puncturing the leaf with a sharp pencil, and the tracing is afterwards measured with a planimeter. If the cup area differs slightly from that for which a curve of $\log m/R_2$ against $\log ms_1$ is available, interpolation between two curves is carried out. This is also resorted to when a curve is not available for the observed ratio of upper to lower stomata. Values of s_1 (conductance per unit area) have been found and the conductances have also been calculated per 10,000 stomata S_s. The latter measure is preferable for many purposes as there are considerable variations in number of stomata per unit area between leaves and even between different parts of the same leaf. Thus in the Pelargonium leaves used the mean number of lower stomata has been found to vary from about 10,000 to 20,000 per cm.^{2}, and sometimes the number varies by as much as 40 per cent. between two positions on the leaf. The upper stomata are even more variable, ranging in different leaves from 0 to about 1,800 per cm.^{2}, and their ratio to the lower stomata ranging from zero to 0·18 but being generally less than 0·10. In *Begonia sanguineum* there are no upper stomata and the lower stomata are far less numerous and less variable in number. Thus in the leaves used the number per cm.^{2} varies between leaves from about 6,300 to 6,800. The maximum variation noted between two positions on the same leaf was 36 per cent.

(c) *Tests of the proportionality of flow and pressure drop.* These tests have been carried out by taking alternate readings with the standard resistances A or B at R_1 (see p. 465, above) during several experiments and the results are presented in Table II. Owing to changing stomatal aperture it is necessary to interpolate for one or the other sets of data, and interpolated values are shown in italics in the table. Logarithms of R_2 have been used in order to equalize the errors as much as possible (see section 1 (d)) and natural logarithms have been preferred in order that the differences (values in column 3 minus values in column 6) may represent a relative change which when multiplied 100 times will give percentage change in R_2. Values of $\log ms_1$ have been tabulated in ascending order for each experiment and it will be seen that the differences in $\log_e R_2$ (column 3 minus column 6) show no general trend with changing stomatal conductance. It should be noted that positive differences correspond to an apparent increase of resistance with increase of (P_1-P_2), such as would occur with turbulent flow, and it will be seen that the mean difference is negative. This mean is shown by the t test (Fisher, 1925–38)

TABLE II

Pelargonium

Tests of Proportionality of Flow and Pressure Drop Across the Leaf

1 unit of resistance $= 3\cdot77 \times 10^8$ cm.$^{-3}$. Interpolated data in italics.
Pressure drop cm. of H_2O.

1. Date of experiment, and cup used.	2. P_1-P_2	3. $\log_e R_2$	4. $\log_{10} ms_1$	5. P_1-P_2	6. $\log_e R_2$	7. $\log_{10} ms_1$	Difference 3–6.
	$R_1 = 0\cdot299$ units.			$R_1 = 1\cdot17$ units.			
3.5.39.	7·40	−0·823	0·44	3·40	−0·741	0·39	−0·082
Leaf chamber	7·16	−0·764	0·41	3·38	−0·753	0·40	−0·011
LI	*6·31*	*−1·051*	*0·58*	2·68	−1·059	0·60	+0·008
(with knife)	3·49	*−2·060*	*1·43*	1·12	−2·096	1·46	+0·036
3.5.39.	8·44	−0·275	1·55	*4·68*	*−0·256*	*1·53*	−0·019
Small circular	8·04	−0·436	1·72	*4·25*	*−0·413*	*1·69*	−0·023
cup.	*7·93*	*−0·479*	*1·78*	4·12	−0·466	1·75	−0·013
	7·26	−0·730	2·01	3·48	−0·714	2·00	−0·016
27.4.39.	*5·30*	*−1·400*	*0·86*	1·96	−1·457	0·90	+0·057
Leaf chamber	5·08	*−1·475*	*0·92*	1·84	−1·528	0·97	+0·053
LI	3·56	*−2·038*	*1·41*	1·34	−1·898	1·29	−0·140
(with knife)	2·78	*−2·375*	*1·68*	0·84	−2·410	1·71	+0·035
27.4.39.	7·60	−0·607	*1·92*	3·86	−0·564	1·86	−0·043
Small circular cup.							
24.10.39.	9·00	*+0·148*	*1·19*	5·72	+0·122	1·21	+0·026
Small circular cup.							
7.10.38.	*8·83*	*−0·178*	*1·30*	5·42	−0·039	1·18	−0·139
Small circular	*8·42*	*−0·348*	*1·44*	4·82	−0·242	1·35	−0·106
cup.							
					Mean difference		−0·024

not to be significantly different from zero ($P > 0\cdot1$); there is thus no evidence of lack of proportionality between flow and pressure drop. The range of $\log ms_1$ in these experiments corresponds to a forty-fold change of stomatal conductance. It should be mentioned that an assumed value of α of 0·04 has been used in obtaining $\log ms_1$ as counts of upper stomata were not obtained.

(d) '*Double cup*' experiments. *Estimation of m and effect of upper stomata.* These experiments had two objects; the first was to determine the resistances with and without an extra length of path through the intercellular spaces so that *m*, the surface resistivity of the mesophyll, could be estimated. The second object in the case of Pelargonium was to investigate the effects of the upper stomata upon the total resistance. Two such experiments have been carried out with Pelargonium and one with Begonia; for these the leaf chambers (Heath, 1939) have been used without the knife. The change in resistance brought about by adding in effect 6 mm. to the width of the washer (a_2-a_1) is found under hypostomatous conditions by comparing the value of R_h when the

upper chambers *UI* and *UO* are closed, with that obtained when the lower outer chamber *LO* is also closed (R'_h say). In the Pelargonium experiments further comparisons have been made between the resistance R_a with all chambers except the porometer cup *LI* open to the outside air, and those R_h under more or less hypostomatous conditions with either the upper inner chamber *UI* alone or both the upper chambers closed. Such comparisons give information as to the effect of the upper stomata on total resistance measured. For the 'extra path' determinations the general procedure is to take readings in the following order, *LI* indicating the porometer cup and closed chambers being shown in brackets: *LI(UI, UO)*; *LI(UI, UO, LO)*; *LI(UI, UO, LO)*; *LI(UI, UO)*. The mean of the two *LI(UI, UO, LO)* determinations can thus be compared with the value for *LI(UI, UO)* inter-polated to the same time. In order to estimate the effects of the upper stomata, other readings are taken in the order: *LI(   )*[1]; *LI(UI)*; *LI(UI)*; *LI(   )* or: *LI(   )*; *LI(UI, UO)*; *LI(UI, UO)*; *LI(   )*. After opening, and especially after closing chambers, it is necessary to make sure that the new state of equilibrium has been reached before taking further readings.

Experiment of 8.5.39. Pelargonium. Clone 5. A leaf of $5\frac{3}{4}$ in. diameter was cut off and attached to a water supply by the method of Gregory (1938) at 10.50 a.m., and was placed in darkness until 1.0 p.m. when it was set up in the leaf chambers. A small cup was fitted on another part of the leaf at 1.30 p.m. (see section 2 (*e*) below). Readings as described above were made from 4.0 p.m. while the stomata closed slightly. At 6.2 p.m. the light (described in the previous paper) was switched on and a further series of similar readings was taken when the stomata had opened considerably. A few small injected patches developed under the washers during the latter part of the experiment. The number of lower stomata within LI was estimated as 11,990 per cm.[2] Unfortunately no counts were made of upper stomata.

Experiment of 12.12.39. Pelargonium. Clone 3. An attached leaf of diameter $4\frac{1}{2}$ in. on a plant grown from a June cutting was set up overnight in the leaf chambers and darkened. Readings as above were carried out from 11.8 a.m. while the stomata remained almost stationary. At 12.12 p.m. the leaf was illuminated by means of a 200 w. lamp, with water screen, about one foot distant and further readings were taken with open stomata. The strips of lower epidermis revealed that in a number of stomata the guard cells had collapsed and were apparently dead. Where such stomata appeared definitely closed and non-functional they were omitted from the count which therefore gave the low value of 7,860 per cm.[2] The upper stomata averaged 330 per cm.[2] giving a ratio of upper to lower of 0·042.

Experiment of 13.12.39. Begonia. A leaf $6\frac{1}{2}$ in. long was cut off and placed in water at 10.15 a.m. It was set up in the leaf chambers with the 200 w. light on and readings were begun at 11.15 a.m. After a series of readings during which the stomata opened somewhat the leaf was darkened at 1.51 p.m. Very little stomatal closure occurred during the afternoon, so a new end was cut on the petiole and the leaf was left darkened overnight. Next morning the stomatal resistance had in-creased considerably and a further series of readings was taken. The number of stomata within *LI* was estimated as 6,383 per cm.[2]

---

[1] Empty brackets indicate that all chambers except *LI* were open to the outside air.

TABLE III

Pelargonium

Double-cup Experiments. Estimation of m, the Surface Resistivity of the Mesophyll

1 unit of resistance $= 3 \cdot 77 \times 10^8$ cm.$^{-2}$.

Date.	$\log ms_1$*	S_s	R_h $LI(UI,$ $UO)$	R'_h $LI(UI,$ $UO, LO)$	$R'_h - R_h$	m
8.5.39.	$\bar{1}\cdot94$	0·15	1·055	1·156	0·101	
	$\bar{1}\cdot94$	0·15	1·060	1·156	0·096	} 5·4
	0·00	0·17	0·955	1·043	0·088	
	1·33	3·64	0·173	0·308	0·135	4·88
	1·39	4·18	0·166	0·299	0·133	4·78
					Mean =	4·83
12.12.39.	0·24	0·45	0·623	0·707	0·084	
	0·27	0·48	0·599	0·669	0·070	} 4·2
	0·28	0·49	0·586	0·674	0·088	
	1·49	8·03	0·152	0·285	0·133	4·75
	1·52	8·60	0·149	0·286	0·137	4·87
	1·54	9·01	0·146	0·286	0·140	4·96
					Mean =	4·86

Begonia

Date.	$\log ms_1$†	S_s	R_h	R'_h	$R'_h - R_h$	m
13.12.39.	0·16	0·14	2·494	2·511	0·017	
	0·17	0·14	2·469	2·503	0·034	} <10
	0·17	0·14	2·443	2·494	0·051	} >4
	0·19	0·15	2·366	2·383	0·017	—
	0·19	0·15	2·330	2·345	0·015	—
	0·93	0·80	0·871	1·206	0·335	—
	0·99	0·92	0·821	1·181	0·360	—
	1·04	1·04	0·765	1·191	0·426	—
	1·08	1·14	0·748	1·184	0·436	—
	1·13	1·27	0·715	1·163	0·448	16·5
	1·19	1·46	0·685	1·129	0·444	16·3
	1·21	1·53	0·658	1·122	0·464	16·9
					Mean =	16·6

* Derived from $\log m/R_h$. To obtain $\log s_1$ subtract 0·69.
† „ „ „ · „ „ „ 1·22.

The results of the above three experiments showing the effect of an extra 6 mm. width of washer are given in Table III. Within each experiment the data have been arranged in ascending order of stomatal conductance, as shown by the values of $\log ms_1$ or S_s (the conductance per 10,000 stomata) tabulated in the second and third columns. The fourth and fifth columns show the resistances R_h and R'_h found under hypostomatous conditions with the lower outer chamber open and closed respectively. The difference $R'_h - R_h$ gives

the additional resistance due to an extra 6 mm. of washer and it will be seen that this rises as the stomata open. This confirms Newton's suggestion that as the stomata open less of the air enters through those remote from the cup, for if less air is *normally* passing across the 6 mm. of extra path the increase in resistance when LO is closed must be greater. It also demonstrates the increasing importance of the effect of the washer with increasing stomatal conductance. The last column of the table gives values of m calculated by the method of successive approximation given in the Appendix (p. 497). This calculation has been carried out for the individual values of R'_h and R_h corresponding to the two or three largest stomatal conductances in each experiment, and means of the values of m so obtained have been taken for actual use. The remarkable agreement between the mean values of m found in the two experiments with Pelargonium will be noted. For the smaller stomatal openings, means of three values of R_h and the three corresponding values of R'_h have been used. Here the errors are larger for $(R'_h - R_h)$ is less accurately determined by means of the porometer. Nevertheless, for Pelargonium the results are in reasonable agreement with those found at larger stomatal apertures and there is no evidence of a consistent trend. The attempt to estimate m at the smallest stomatal conductances for Begonia failed owing to the lack of sensitivity of the porometer in terms of R at such high resistance. It will be noted that the internal resistance of the Begonia leaf is considerably higher than that for Pelargonium.

Table IV gives the results of the two experiments with Pelargonium showing the effect of the upper stomata. As in Table III, the second and third columns show values of log ms_1 and S_s arranged in ascending order. For the experiment of 8.5.39 a stomatal ratio of 0·04 has been assumed. The resistances R_a under amphistomatous conditions are shown in the fourth column while those R_h with one or both of the upper chambers closed are given in the fifth. The difference $R_h - R_a$ and the ratio R_h/R_a given in the last two columns show respectively the absolute and relative effects upon resistance of stopping flow through the upper stomata, either above the cup only $[LI(UI)]$ or over a wider area $[LI(UI, UO)]$. It will be seen that there are no consistent differences between the results obtained with one or with both of the upper chambers closed, indicating that the effect of the upper stomata outside the area immediately above the cup is relatively unimportant. This might be expected on theoretical grounds. The conditions with the upper chambers closed may therefore be considered as approximating closely to those for a hypostomatous leaf, incidentally a requisite condition for the evaluation of m. It is apparent from the results in Table IV that the effect of the upper stomata increases greatly at high stomatal conductances, as is predicted by theory. It will be noted that in the experiment of 8.5.39 the last two values of R_h show a slight increase while R_a is still falling. This may perhaps be attributed to the development of the injected patches referred to above (p. 480). These results are considered further in the Discussion below.

TABLE IV

Pelargonium

Double-cup Experiments. Effect of Upper Stomata

1 unit of resistance $= 3\cdot77 \times 10^8$ cm.$^{-3}$

Values in italics indicate that R_h was obtained with only the upper inner chamber closed $[LI(UI)]$.

Date.	log ms_1*	S_s	R_a LI( )	R_h LI(UI) or LI(UI, UO)	$R_h - R_a$	R_h/R_a
8.5.39.	1̄·93	0·145	1·029	*1·045*	0·016	1·02
	1̄·94	0·148	1·025	*1·034*	0·009	1·01
	1̄·95	0·152	1·019	*1·045*	0·026	1·03
	0·02	0·178	0·872	0·883	0·011	1·01
	0·08	0·204	0·791	*0·808*	0·017	1·02
	1·94	14·8	0·0652	*0·1591*	0·094	2·44
	1·97	15·9	0·0636	0·1609	0·097	2·53
	2·03	18·2	0·0577	*0·1797*	*0·122*	3·11
12.12.39.	0·23	0·44	0·617	0·623	0·006	1·01
Ratio of	0·25	0·46	0·596	*0·605*	0·009	1·02
upper/lower	0·25	0·46	0·592	0·601	0·009	1·02
stomata =	0·26	0·47	0·581	0·586	0·005	1·01
0·042	0·29	0·51	0·555	*0·556*	*0·001*	1·00
	1·36	6·0	0·137	*0·153*	0·016	1·12
	1·39	6·4	0·132	0·152	0·020	1·15
	1·47	7·7	0·120	0·149	0·029	1·24
	1·48	7·9	0·118	*0·148*	0·030	1·25
	1·53	8·8	0·112	0·146	0·034	1·30
	1·54	9·0	0·111	*0·144*	0·033	1·30
	1·58	9·9	0·106	*0·144*	0·038	1·36

* These figures are derived from $\log m/R_a$, assuming $\alpha = 0\cdot04$. To obtain $\log s_1$ subtract 0·69.

(e) *Comparison of leaf chamber and small circular cup.* Three experiments have been carried out with Pelargonium in which alternate readings have been taken with the leaf chamber LI as porometer cup on one part of a leaf and a small circular porometer cup on another part of the same leaf. In two of these the knife has been in position round the side of the outer leaf chambers nearest the edge of the leaf.

Experiment of 27.4.39. Pelargonium. Clone 5. A leaf of $5\frac{1}{4}$ in. diameter was cut off and attached to a water supply at 10.15 a.m. After being placed in the dark and left until 1.0 p.m. it was set up in the leaf chambers (with knife) and again darkened. A small cup was fixed on another part of the leaf at 4.0 p.m. and gave a very high reading at once. Readings were carried out from 4.40 p.m., the leaf being illuminated after the first twenty minutes. The estimated numbers of lower stomata were 11,570 per cm.² for the leaf chamber and 10,870 per cm.² for the small cup.

Experiment of 3.5.39. Pelargonium. Clone 5. A leaf of $5\frac{1}{4}$ in. diameter was attached to a water supply at 9.50 a.m. and set up in the leaf chambers with the knife in position at 10.15 a.m. The small cup was fitted at 11.15 a.m., but apart from this the leaf was kept in the dark until and after readings were begun at 12.11 p.m.

The leaf was illuminated after the first $38\frac{1}{2}$ minutes of readings, and the stomata opened steadily up to 100 minutes when the leaf was again darkened. Further readings were taken while the stomata closed. The numbers of lower stomata were estimated as 10,280 per cm.² and 11,830 per cm.² for the leaf chamber and small cup respectively.

Experiment of 8.5.39. Pelargonium. Clone 5. The setting up of this experiment has already been described above (p. 480). A number of readings was taken with the small cup, while the leaf was in darkness, for comparison with the leaf-chamber results and a series of alternate readings was taken following the switching on of the light. The lower stomata within the small cup were estimated as numbering 14,870 per cm.²

The results of these three experiments are presented graphically in Fig. 8. Values of S_s, the conductance per 10,000 stomata, have been calculated and their logarithms plotted against time. Unfortunately, no counts of upper stomata were made for any of these experiments, and an assumed value of α of 0·04 has been used in obtaining $\log ms_1$ from $\log m/R_2$ by means of the theoretical curves. The other constants used are indicated in the figures, except m which in all cases has been taken as 4·85.

It will be seen that there is some general agreement between the values of $\log S_s$ for the leaf chamber and small cup. During the preliminary period of darkness the stomatal behaviour is somewhat erratic. This might be due to a variety of causes such as incomplete recovery from the shock of fixing the cup, or slight leakages of light. It is probable that not much significance should be attached to the difference between the stomatal conductance for the leaf chamber and that for the small cup during this period. All three experiments agree, however, in showing at the widest stomatal apertures a somewhat higher stomatal conductance obtained from the leaf-chamber data than from the small cup. The discrepancy is no more marked with the knife (27.4.39 and 3.5.39) than without it (8.5.39) and it seems that the use of $b = 2·0$ under the former conditions is a sufficiently near approximation in the present state of accuracy of the method. The difference could be reduced but by no means annulled by assuming a much higher value of α. Thus the difference of the final values of $\log S_s$ in the 8.5.39 experiment is 0·50 (corresponding to a factor of 3 for S_s). If α is taken as 0·10, this difference is reduced to 0·34 (a factor of 2·2). Similarly, even doubling the value used for m only reduces this difference in $\log S_s$ to 0·21 (a factor of 1·6). One point in connexion with the technique should be mentioned since it will tend to cause differences in the observed sense. With the small cup, the leaf is pressed lightly against a glass or perspex plate and hence there must be some resistance to flow of air to the upper stomata. It seems improbable that this resistance will be appreciable compared with that of the upper stomata in view of the virtual impossibility of making an air-tight joint on the hairy epidermis even with a gelatine washer unless it is greased. However, such resistance as does arise from this cause will be additional to that of the upper
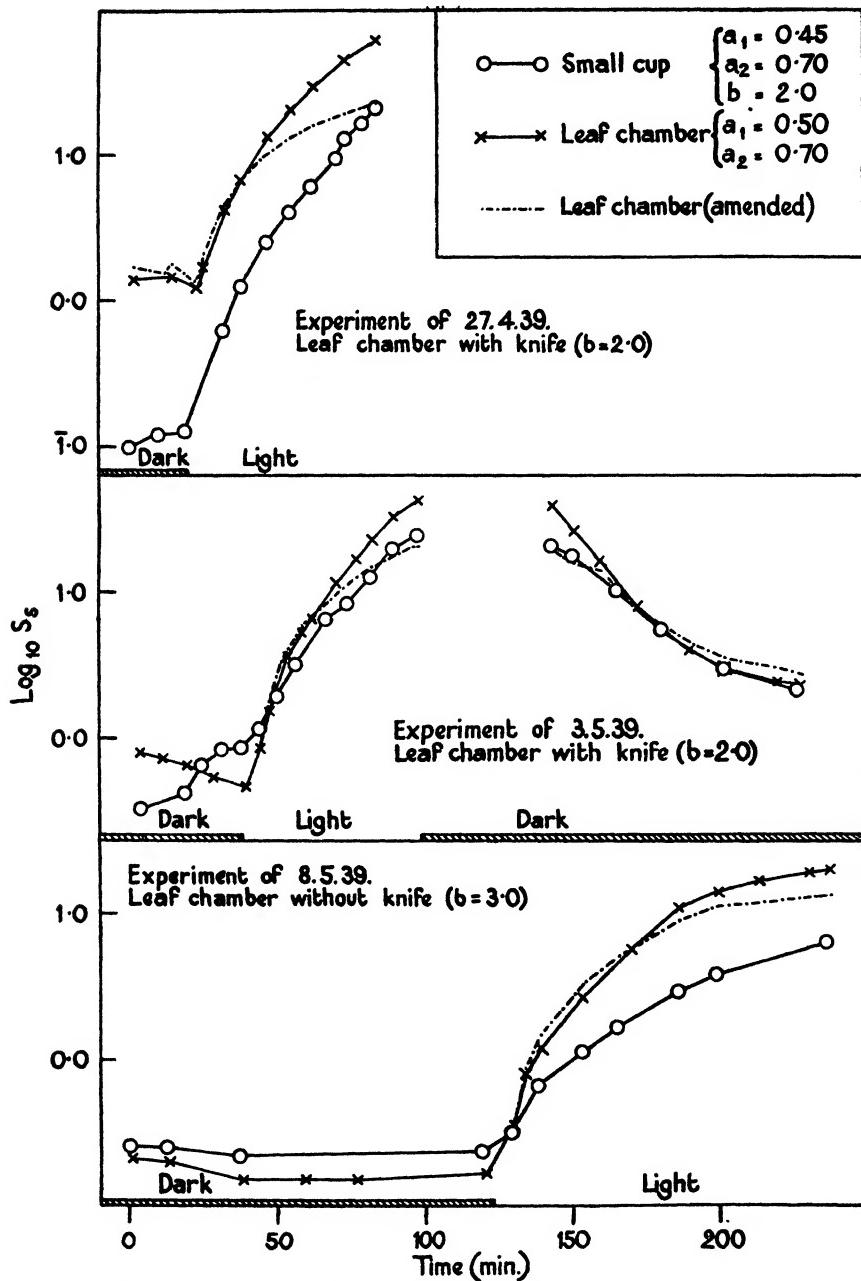
FIG. 8. Pelargonium. Comparison of the values obtained with the leaf chamber *LI* and a small circular cup during stomatal movement. Logarithms of conductance S_s per 10,000 stomata of the lower surface.

stomata and hence will have an effect somewhat similar to a reduction in their number. No such resistance occurs in the case of flow to the upper stomata above *LI*. Considering again the final values in the experiment of date 8.5.39 it would be necessary to assume $\alpha = 0\cdot10$ for the leaf chamber and $\alpha = 0\cdot00$ for the small cup in order to remove the whole of the discrepancy. It would appear that the difference between S_s for *LI* and for the small cup must be attributed at least in part either to actual differences in stomatal aperture or to inadequacy in the theory for the elongated cup, since this is certainly much less precise than that for a circular cup. The consistent differences found at wide apertures in all three experiments suggest that the latter is more likely to be the cause. This question is considered further in the Discussion below, where an explanation of the 'amended values' in Fig. 8 will be found.

3. *Discussion*

The conclusions to be drawn from the available experimental evidence bearing upon the adequacy of the theory for an elongated cup may now be considered. The experiments in which the leaf chambers are used as a double cup to estimate m for Pelargonium under hypostomatous conditions agree in giving an approximately constant value for m over a considerable range of stomatal conductance. This range is equivalent to a change in log ms_1 from $0\cdot0$ to at least $1\cdot5$ (Table III), and if log ms_1 is calculated from m/R_a with an assumed value for α of $0\cdot04$ (as in Table IV) the uppermost value of log ms_1 is estimated as $2\cdot0$. This constancy of m would appear to provide evidence that the *shape* of the $\alpha = 0$ curve relating log m/R_2 to log ms_1 is approximately correct, although as mentioned earlier (p. 476) the existence of an error acting as a multiplying constant may displace the curve.

The second test suggested in section 1 (*f*) is illustrated in Fig. 9. At chosen values of log ms_1 the differences log m/R_a—log m/R_h (i.e. log R_h/R_a) have been read off from the theoretical curves relating log m/R_2 to log ms_1 for several values of α. These differences are plotted against log m/R_a to give a series of theoretical difference-curves. From the data for the two experiments with Pelargonium in which the effect of the upper stomata was investigated, observed values of log R_h/R_a have also been plotted against log m/R_a using the value of m actually found for these leaves, namely $4\cdot85$. Since the observed points from both experiments seem to lie upon a smooth curve, a single free-hand curve has been drawn through them. The observed stomatal ratio for the experiment of 12.12.39 is $0\cdot04$, that for the experiment of 8.5.39 being unknown but likely to be similar. It will be seen that there is a similarity both of form and position between the observed and calculated curves which provides some general confirmation of the theory, especially as the test is obviously an extremely sensitive one. But since the divergence from theory is evidently real it is necessary to inquire which of the many assumptions involved in the theory may be at fault. Neglect of the end effects in the leaf
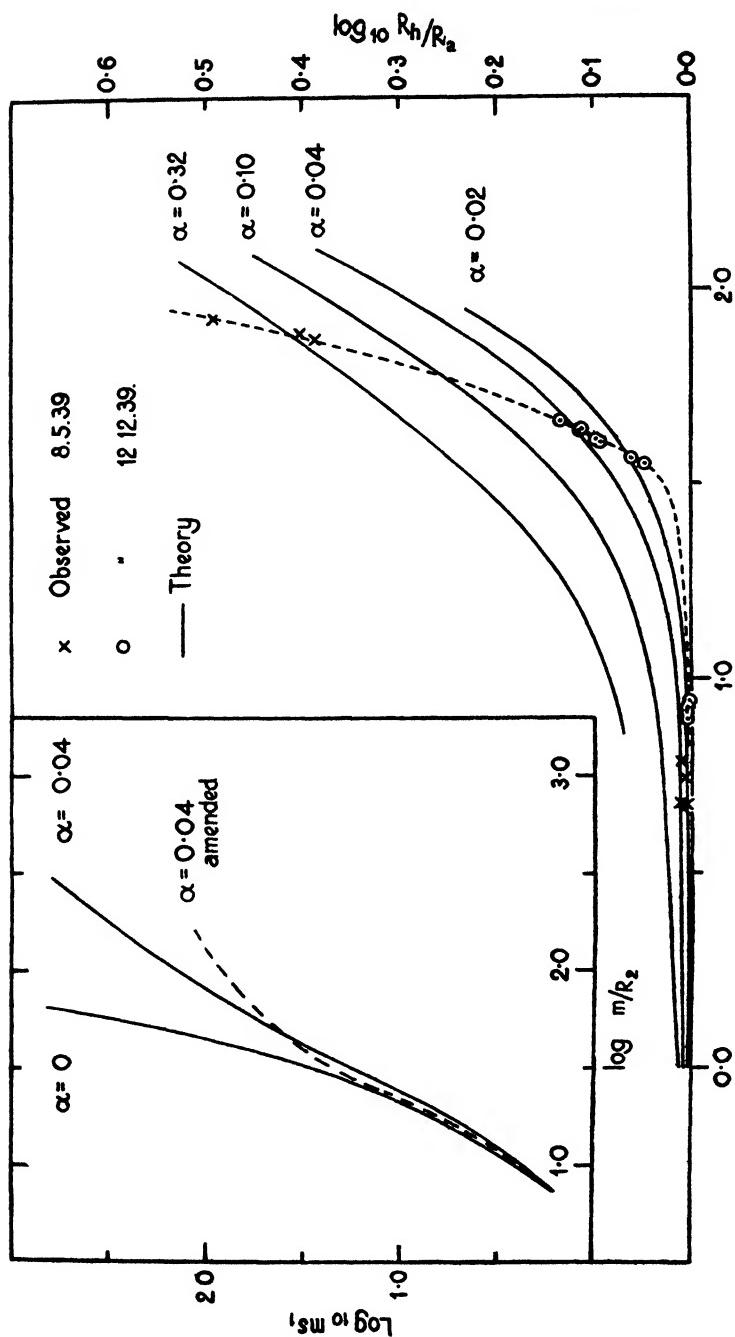
FIG. 9. For explanation see text.

chamber will mean that at wide stomatal apertures, when the washer has much more effect upon $\log m/R_h$ than upon $\log m/R_a$, the theoretical values of $\log R_h/R_a$ are too large. Correcting this error, which is unlikely to be a large one since the width of LI is only 11 per cent. of the length, would make the fit even worse. Even large errors in b have practically no effect at medium and large stomatal apertures. Errors in m, if constant, would affect the position but not the shape of the observed curve. It has been shown experimentally that m is approximately constant over the range of stomatal conductance under discussion. If the resistance to flow through the palisade tissue was appreciable, this would be additional to the resistance of the upper stomata and hence of most importance at large stomatal conductances. It would then have an effect similar to a decrease in α. Allowance for this would make the corrected theoretical difference-curves lower and the fit worse. There is, however, one assumption which if incorrect might cause a deviation from theory in the direction observed, namely that the ratio α of the conductances s_1 and s_2 is constant and that it is the same as the ratio of the stomatal numbers. Should the upper stomata open in the light much more rapidly than the lower, owing to the higher light intensity or difference in quality, α would increase as the stomata opened. This would give a difference in the curves similar to that found, e.g. suppose that in the experiment of 8.5.39 the stomatal ratio were 0·04 and that α had this value in the dark at $\log m/R_a = 0·7$. If the upper stomata were to open more rapidly in the light, so that at a certain stage they were twice as widely open as the lower, their relative conductance might be expected to increase about 2^3 times and α would now be 0·32. A test of this hypothesis could be carried out by repeating the experiments with illumination from below instead of from above. If the lower stomata then opened more rapidly than the upper, the observed curve should have a trend away to the right of the theoretical one. It is hoped to make this test at a future date, but it must be pointed out that, with either type of illumination, as both upper and lower stomata approach their full aperture the observed curve should first turn back towards the theoretical curve and ultimately run parallel with it. No tendency for this to occur is shown in the present experiments, despite the fact that in both cases the stomata were almost stationary after a long period of illumination when the observations at the widest apertures were made. In the case of the experiment of 8.5.39 the lack of tendency for the curve to bend over might be attributed to the small injected patches under the washer (see pp. 480 and 482) which would tend to increase R_h/R_a. No such explanation is applicable to the experiment of 12.12.39. The assumption referred to above as a possible cause of the discrepancy between observation and theory implies that interference between the stream-lines flowing through neighbouring stomata is negligible. Such interference will not only increase in amount as the stomata open and are therefore separated by fewer diameters, but it will certainly be much more important for the lower than for the widely separated upper

stomata. Its effect if appreciable will be to cause an increase of α as the stomata open, thus tending to give the observed result. This explanation, however, seems unlikely to apply to the discrepancy between the results for the leaf chamber and a small cup discussed in the next paragraph. It will be appreciated that it is by no means obvious where the inadequacy lies in the theory for an elongated cup. As indicated in the previous paragraph, the theory for a hypostomatous leaf would appear to be either adequate or else invalid owing to a multiplying constant only (e.g. an error in the effective length of the chamber l). It is apparent, however, that the theory for an amphistomatous leaf would be improved by being amended in some respects. Some indication of the type of amendment needed is shown in the inset to Fig. 9. Here parts of the theoretical $\alpha = 0$ and $\alpha = 0.04$ curves relating $\log m/R_2$ to $\log ms_1$ are shown for the leaf chamber LI ($a_1 = 0.5$; $a_2 = 0.7$; $l = 9.0$; $b = 3.0$). Assuming the $\alpha = 0$ curve to be the more reliable, values of $\log R_h/R_a$ have been read off from the observed curve at chosen values of $\log m/R_a$ and added to the values on the calculated $\alpha = 0$ curve at the same $\log ms_1$. Hence an amended $\alpha = 0.04$ curve (shown as a broken line) has been obtained for $\log m/R_2$ v. $\log ms_1$. The part of this curve where $\log m/R_2$ is greater than 1.7 should be regarded with caution, depending as it does upon those results of the 8.5.39 experiment in which injection may have affected the observed value of $\log R_h/R_a$.

The experiments comparing stomatal conductances obtained with the leaf chamber and small cup respectively also indicate that the theory for an elongated cup needs some amendment, if it is assumed that the theory for a circular cup is correct. The type of correction apparently needed is such as would be given by an increasing value of α as the stomata open, i.e. the type of amended curve shown in the inset to Fig. 9. It should be noted that here the discrepancy is unlikely to be caused by interference between flow through neighbouring stomata, or by the upper stomata opening more rapidly in the light, for these factors should affect similarly both the small cup and leaf chamber results. Making use of the amended curve (Fig. 9), the values of $\log S_e$ for the leaf chamber have been recalculated for each of the three experiments and curves of amended values plotted in Fig. 8. It will be noted that in all cases there is a marked improvement in the agreement at large apertures with the results for the small cup, indicating that this is approximately the type of correction needed. For the present, however, the theory for the elongated cup will continue to be used in its original form, which as the experimental tests show is a useful approximation.

It is realized that the experimental data are very meagre considering the extent of theory involved, and much further work will be needed. The paucity of data is the result of the experimental work being carried out before the theoretical aspects of the problem were elucidated. The fortunate collaboration of Dr. Penman eventually made this possible, but at a time when further experimentation was interrupted by the war.

SUMMARY

A theoretical study of the resistance porometer method is presented, together with the results of an experimental investigation of the adequacy of the theory formulated. The theoretical considerations are first dealt with and the experimental evidence is later presented and discussed. The mathematical treatment of the theory is presented in an appendix by Dr. H. L. Penman.

Previous attempts to calibrate porometers are briefly reviewed.

The structure of the leaves used (*Pelargonium zonale* and *Begonia sanguineum*) is described with reference to the paths of flow of gas during porometer experiments.

The methods of calculation of flow resistance or conductance from porometer readings are described, the conditions for their valid use are considered, and the theoretical limitations involved are discussed in detail.

The sensitivity of the resistance porometer is considered, and it is concluded that from a statistical point of view the use of logarithmic values of resistance and conductance is to be preferred.

The total resistance or conductance as estimated with the porometer is analysed into that due to (1) the stomata within the cup and (2) the mesophyll and the remaining stomata. Previous work along these lines is discussed. The consequences of the theoretical treatment of this relation are considered (*a*) for a circular porometer cup and (*b*) for elongated cups. For the latter the theory is not precise and therefore experimental tests of its validity are given.

The principal condition necessary for the valid estimation of leaf resistance is proportionality between flow and pressure difference. Experimental confirmation of this condition is presented.

By the use of the leaf chambers (Heath, 1939) as a 'double cup' the value of the surface resistivity (*m*) of the mesophyll is estimated. These experiments also confirm certain predictions based on the theoretical investigation, namely the increasing importance with stomatal opening of the width of the washer attaching the porometer cup to a hypostomatous leaf, and also to some extent the form of the theoretical curve relating total resistance and stomatal conductance for such a leaf.

During these experiments with the leaf chambers the effects of the upper stomata upon total resistance have been investigated by opening and closing the upper chambers to the outer air. In this way the increasing importance of the upper stomatal effect with increasing aperture is demonstrated, thus confirming another prediction from the theoretical investigation. It is shown that with the upper chambers closed the conditions approximate closely to those for a hypostomatous leaf, this being a requisite condition for the estimation of *m*.

Experiments with the leaf chambers and a small circular porometer cup on the same leaf show that there is general agreement in the values of stomatal conductance obtained from the respective theories for these two types of cup.

Nevertheless, the results at wide stomatal apertures indicate that the theory for an elongated cup requires some amendment.

The relevant experimental evidence for the need of such amendment and the type of correction required are discussed.

APPENDIX

Theory of Viscous Flow Porometers

BY

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Each of the experimental systems requires separate treatment. The theory of the circular cup, used also for diffusion, is being published elsewhere, and the first part of this appendix is a very brief sketch of the theory of viscous flow; it consists essentially of the equations needed for calculation, and little else. For the leaf chambers more detail is supplied, and the analysis given for these will indicate the principles and methods used in obtaining the results for the circular cup.

I. *Theory of viscous flow with circular porometer cup.*

The leaf, or part of the leaf, is assumed to be circular and bounded by an impervious wall around the circumference. Stomatal perforations in each of the surfaces permit the flow of fluids through the epidermis and it is assumed that mean sizes of the two sets are equal, and that mutual interference between the stream-lines of flow through neighbouring stomata is either non-existent or affects both sets equally; hence the ratio of the numbers per unit area gives the conductivity ratio. The porometer cup is supposed applied symmetrically about the centre of the lower epidermis. The leaf is supposed so thin that there is no resistance to flow across the thickness, but there is resistance to flow within the leaf parallel to the plane of the leaf. It is assumed that pressure gradients are everywhere small so that changes in density can be neglected. In any self-consistent set of units, let

b = radius of disc of leaf (cm.),
a_2 = radius of outer edge of cup,
a_1 = radius of inner edge of cup,

s_1 = conductivity of lower epidermis (per sq. cm.),
s_2 = conductivity of upper epidermis (per sq. cm.),
m = superficial resistivity of mesophyll (per cm./cm.),
p_0 = pressure difference between outside air and inside cup,
i = flow produced (per sec.),
R = measured resistance,
p = difference between pressure at radius r inside the leaf and that inside the cup.

Flow equation. By considering an annular ring, radius r width δr, flow
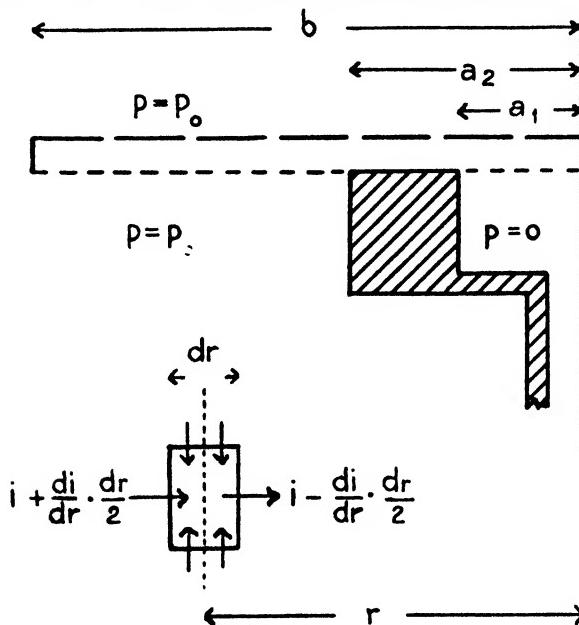
FIG. 10. Idealized half section of leaf and porometer cup.

equations for the parts of the leaf (i) outside the cup, (ii) inside the cup, and (iii) under the washer, can be set up. These are all of the same form and the solutions are:

$$b > r > a_2, \qquad p_0 - p = AI_0(\rho_1) + BK_0(\rho_1), \qquad (1)$$

$$a_1 > r > 0, \qquad \frac{s_2 p_0}{s_1 + s_2} - p = CI_0(\rho_1) + DK_0(\rho_1), \qquad (2)$$

$$a_2 > r > a_1, \qquad p_0 - p = FI_0(\rho_2) + GK_0(\rho_2), \qquad (3)$$

where
$$\rho_1 = r\sqrt{\{m(s_1 + s_2)\}} = r\beta_1, \quad \text{say,}$$

$$\rho_2 = r\sqrt{(ms_2)} = r\beta_2,$$

$I_0(\rho)$ and $K_0(\rho)$ are Bessel functions,[1] and $A, B, C, D, F,$ and G are constants

[1] The tables employed in this part of the work are B.A. Mathematical Tables; Bessel Functions, Part I (Cambridge Univ. Press, 1937).

to be determined from boundary conditions. These boundary conditions are: (i) at $r = 0$, $p \neq \infty$; (ii) at $r = b$ there is no flow across the boundary; (iii)–(vi) at $r = a_2$, and $r = a_1$, the values of p and dp/dr are continuous.

Applying these conditions we obtain:

$$\frac{\beta_2}{\beta_1}\left[\frac{K_1(\beta_1 b)I_0(\beta_1 a_2)+I_1(\beta_1 b)K_0(\beta_1 a_2)}{K_1(\beta_1 b)I_1(\beta_1 a_2)-I_1(\beta_1 b)K_1(\beta_1 a_2)}\right] = \frac{(F/G)I_0(\beta_2 a_2)+K_0(\beta_2 a_2)}{(F/G)I_1(\beta_2 a_2)-K_1(\beta_2 a_2)}, \quad (4)$$

$$\frac{\beta_2}{\beta_1}\left[\frac{CI_0(\beta_1 a_1)+\dfrac{s_1 p_0}{s_1+s_2}}{CI_1(\beta_1 a_1)}\right] = \frac{(F/G)I_0(\beta_2 a_1)+K_0(\beta_2 a_1)}{(F/G)I_1(\beta_2 a_1)-K_1(\beta_2 a_1)}. \quad (5)$$

Thus for any assumed value of β_1, given the dimensional constants b, a_1, and a_2, and the ratio s_2/s_1 ($= \alpha$, say), the value of C can be found from the second of these equations, the first being used to evaluate F/G.

The total flow through the cup, i, is given by p_0/R, where R is the measured resistance. We find

$$i = \frac{p_0}{R} = \frac{2\pi s_1}{m(s_1+s_2)}\left[\frac{s_2 p_0}{s_1+s_2}\frac{\beta_1^2 a_1^2}{2} - C\beta_1 a_1 I_1(\beta_1 a_1)\right]. \quad (6)$$

Actually, for computational purposes, we can substitute from (5) in (6). Putting the right-hand side of (5) equal to N, we obtain

$$\frac{m}{R} = \frac{2\pi\beta_1 a_1}{(1+\alpha)^2}\left[\frac{\alpha}{2}\beta_1 a_1 + \frac{1}{\dfrac{I_0(\beta_1 a_1)}{I_1(\beta_1 a_1)} - N\sqrt{\left(\dfrac{1+\alpha}{\alpha}\right)}}\right]. \quad (6a)$$

Also $\beta_1^2 = m(s_1+s_2)$, i.e. $ms_1 = \beta_1^2/(1+\alpha)$. Thus for any assumed value of β_1 and α, values of m/R and ms_1 can be calculated.

Special case. When $\alpha = 0$ (hypostomatous conditions), several terms in (4) and (5) become either zero or infinity. In this case, the solution is a little simpler, and putting the large square bracket of the left-hand side of (4) equal to M, we obtain

$$\frac{2\pi R}{m} = \log_e\frac{a_2}{a_1} - \frac{M\dfrac{a_1}{a_2} - \dfrac{I_0(\beta_1 a_1)}{I_1(\beta_1 a_1)}}{\beta_1 a_1}. \quad (7)$$

The curves of Fig. 11 have been derived in this way, using equations (4), (5), and (6) for $\alpha = 0.04$ and $\alpha = 0.10$, and using equation (7) for $\alpha = 0.00$. The values of β_1 used are such that $\beta_1 a_1 = 0.05$, 0.20, 0.80, 1.60, 3.20, 6.40, 12.80, and 20.0. Other constants used in Fig. 11 are $b = 2.0$ cm.; $a_2 = 0.85$ cm., $a_1 = 0.45$ cm. To obtain a value of s_1 for an observed R it is necessary to know m.

Limiting values. (i) *Small stomatal aperture.* For all small values of α,

including $\alpha = 0$, we find that if $b^2 - a_2^2$ is large compared with a_1^2, then, as $\beta_1 \rightarrow 0$,

$$1/R \rightarrow \pi a_1^2 s_1, \tag{8}$$

or all the resistance is due to the lower epidermis under the cup.
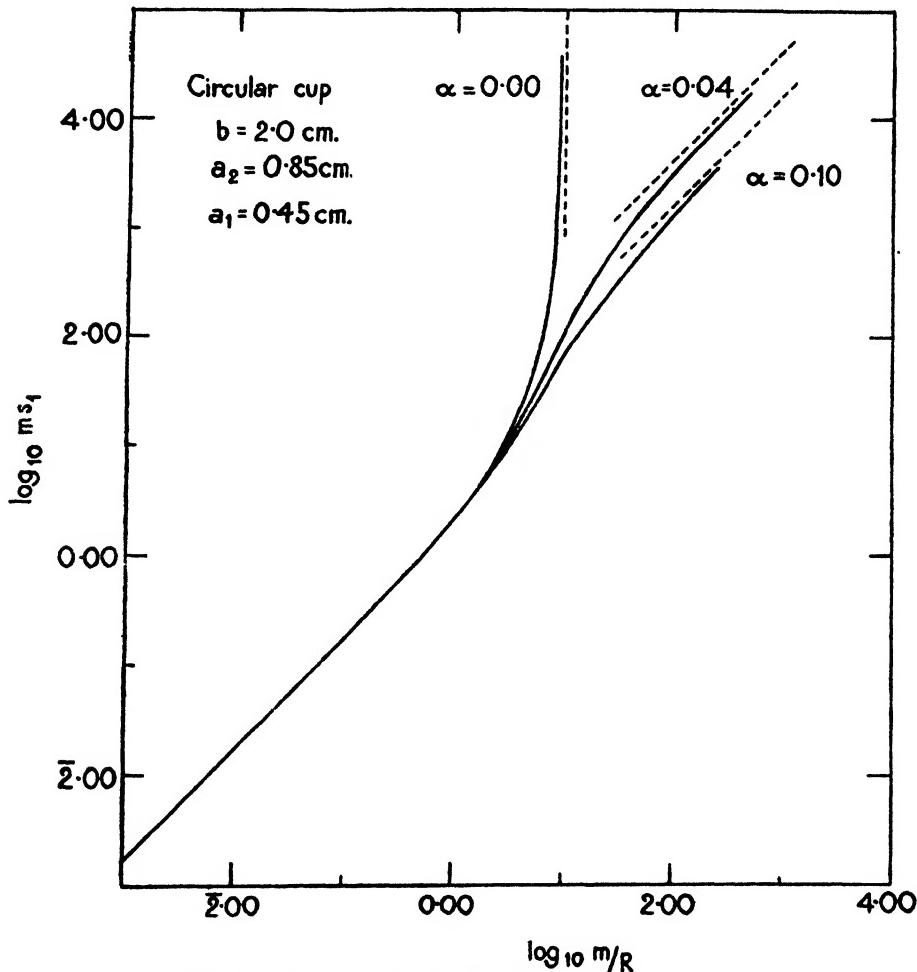
FIG. 11. The relation between total resistance R and stomatal conductance s_1.

(ii) *Large stomatal aperture.* When $\alpha \neq 0$, as $\beta_1 \rightarrow \infty$, we find

$$R \rightarrow \frac{1}{\pi a_1^2 s_1} + \frac{1}{\pi a_1^2 s_2}, \tag{9}$$

i.e. the resistance is wholly due to the resistances of the upper and lower parts of the leaf under the cup. When $\alpha = 0$, we obtain from equation (7), as $\beta_1 \rightarrow \infty$

$$R \rightarrow \frac{m}{2\pi} \log_e \frac{a_2}{a_1}. \tag{10}$$

The asymptotes given by (9) and (10) have been included in Fig. 11.

Determination of mesophyll resistance. To determine m let us suppose that the upper epidermis is closed ($s_2 = 0$) and everything kept constant except a_2 which is increased to a_3. With an obvious symbolism, M_2 becomes M_3, R_2 becomes R_3, and putting $I_0(\beta_1 a_1)/\beta_1 a_1 I_1(\beta_1 a_1) = \gamma$, we have from (7),

$$R_2 = -\frac{m}{2\pi}\left[\frac{M_2}{a_2\beta_1} - \gamma - \log_e\frac{a_2}{a_1}\right],$$

$$R_3 = -\frac{m}{2\pi}\left[\frac{M_3}{a_3\beta_1} - \gamma - \log_e\frac{a_3}{a_1}\right],$$

$$R_3 - R_2 = -\frac{m}{2\pi}\left[\frac{1}{\beta_1}\left(\frac{M_3}{a_3} - \frac{M_2}{a_2}\right) - \log_e\frac{a_3}{a_2}\right]. \tag{11}$$

Assume, as a first approximation, that the first term in the bracket is negligible: we have an approximate value of m, m_1 say, $= \dfrac{2\pi(R_3 - R_2)}{\log_e a_3/a_2}$. Using this value of m_1 and the known value of R_2, $m_1 s_1 (= \beta_1^2)$ can be read from the curve for $\alpha = 0$, i.e. values of $\beta_1 a_1$, $\beta_1 a_2$, and $\beta_1 a_3$ can be found. Thus values of M_3 and M_2 can be estimated, leading to a more exact estimate of m. Further discussion of the importance of m and an alternative method of measuring it will be found below (p. 498).

II. *Theory of viscous flow with leaf chambers* (Heath, 1939, *p.* 476).

The shape of the system involved here does not possess any centre of symmetry similar to the circular cup porometer and an equally rigorous analysis is not possible. It is proposed to treat it as a rectangle of length equal to the area/width ignoring end effects. This can be partly justified for the following reasons: (1) For the inner cup at least, the length is great compared with the width; (2) veins in the leaf generally cross the walls normally, i.e. the predominant flow into the cup is normal to the walls and we can regard the system as a series of approximately rectangular systems separated by veins preventing flow parallel to the main walls.

The symbolism of the preceding section will be used again, with the modification that a_1 becomes the half width of the cup, and the section of Fig. 10 is now to be regarded as that of a strip of length l normal to the plane of the paper. The elementary area is $l\delta x$, and the general expression for the current at any distance x is $i = (l/m)(dp/dx)$. With these modifications the formal equation becomes

$$\frac{d^2\phi}{dx^2} = \beta^2\phi,$$

and in the appropriate ranges we have as solutions,

$$b > x > a_2, \qquad p = p_0 - (Ae^{\beta_1 x} + Be^{-\beta_1 x}),$$

$$a_2 > x > a_1, \qquad p = p_0 - (Fe^{\beta_2 x} + Ge^{-\beta_2 x}),$$

$$a_1 > x > 0, \qquad p = \frac{p_0 s_2}{s_1 + s_2} - (Ce^{\beta_1 x} + De^{-\beta_1 x}).$$

At $x = 0$ we assume that $dp/dx = 0$, leading to $C = D$. The other boundary conditions are as before, namely $dp/dx = 0$ at $x = b$, and continuity of p and dp/dx at $x = a_2$ and $x = a_1$. We obtain

$$Ae^{\beta_1 b} - Be^{-\beta_1 b} = 0,$$

$$Ae^{\beta_1 a_2} + Be^{-\beta_1 a_2} = Fe^{\beta_2 a_2} + Ge^{-\beta_2 a_2},$$

$$\beta_1(Ae^{\beta_1 a_2} - Be^{-\beta_1 a_2}) = \beta_2(Fe^{\beta_2 a_2} - Ge^{-\beta_2 a_2}),$$

$$C(e^{\beta_1 a_1} + e^{-\beta_1 a_1}) + \frac{p_0 s_1}{s_1 + s_2} = Fe^{\beta_2 a_1} + Ge^{-\beta_2 a_1},$$

$$\beta_1 C(e^{\beta_1 a_1} - e^{-\beta_1 a_1}) = \beta_2(Fe^{\beta_2 a_1} - Ge^{-\beta_2 a_1}),$$

and $\qquad i = p_0/R = \int_{-a_1}^{a_1} ls_1 p \, dx = ls_1 \left[\dfrac{2a_1 p_0 s_2}{s_1 + s_2} - \dfrac{2C}{\beta_1}(e^{\beta_1 a_1} - e^{-\beta_1 a_1}) \right].$

Putting $\qquad e^{\theta} + e^{-\theta} = 2 \cosh \theta, \quad$ and $\quad e^{\theta} - e^{-\theta} = 2 \sinh \theta,$

the general solution of these equations can be taken a little further than the corresponding earlier set and we obtain

$$\frac{\beta_2}{\beta_1} \frac{2C \cosh \beta_1 a_1 + \dfrac{p_0 s_1}{s_1 + s_2}}{2C \sinh \beta_1 a_1} = -N, \quad \text{say,}$$

$$= -\frac{\beta_2 \cosh \beta_1(b - a_2)\cosh \beta_2(a_2 - a_1) + \beta_1 \sinh \beta_1(b - a_2)\sinh \beta_2(a_2 - a_1)}{\beta_2 \cosh \beta_1(b - a_2)\sinh \beta_2(a_2 - a_1) + \beta_1 \sinh \beta_1(b - a_2)\cosh \beta_2(a_2 - a_1)},$$

evaluation of which for an assumed value of β_1, and known values of α, b, a_2, and a_1, merely involves a straightforward use of standard tables of sinh and cosh. Knowing N, we have

$$p_0/R = ls_1 \left[\frac{2a_1 p_0 s_2}{s_1 + s_2} - \frac{2C}{\beta_1} 2 \sinh \beta_1 a_1 \right]$$

or $\qquad 1/R = \dfrac{2ls_1}{(1 + \alpha)} \left[\alpha a_1 + \dfrac{\beta_2}{\beta_1} \dfrac{1}{\dfrac{\beta_2}{\beta_1} \coth \beta_1 a_1 + \beta_1 N} \right].$

This expression can be reduced to

$$1/R = \frac{2l}{m(1 + \alpha)^2} \left[\alpha \beta_1^2 a_1 + \frac{\beta_2}{\dfrac{\beta_2}{\beta_1} \coth \beta_1 a_1 + N} \right], \tag{12}$$

giving an expression for m/R for each assumed value of β_1. Also

$$\beta_1^2 = m(s_1+s_2) = ms_1(1+\alpha),$$

i.e.
$$ms_1 = \beta_1^2/(1+\alpha).$$

Special case: α (or β_2) $= 0$. As $\beta_2 \to 0$ the value of N approaches

$$\frac{\beta_2}{\beta_1} \coth \beta_1(b-a_2)+\beta_2(a_2-a_1),$$

and we have, for $\beta_2 \doteqdot 0$,

$$1/R = \frac{2l}{m(1+\alpha)^2}\left[\alpha\beta_1^2\,a_1+\frac{\beta_1\beta_2}{\beta_2\{\coth\beta_1\,a_1+\coth\beta_1(b-a_2)+\beta_1(a_2-a_1)\}}\right],$$

i.e. for $\alpha = 0$ we obtain

$$1/R = \frac{2l}{m}\left[\frac{\beta_1}{\coth\beta_1\,a_1+\coth\beta_1(b-a_2)+\beta_1(a_2-a_1)}\right], \quad \text{where} \quad \beta_1^2 = ms_1. \quad (13)$$

Limiting values. (i) *Small stomatal aperture.* We find for all values of α that as $\beta_1 \to 0$

$$m/R \to \frac{2ls_1\,m}{(1+\alpha)}\left[\alpha a_1+\frac{1}{\dfrac{1}{a_1}+\dfrac{1}{b-a_2}}\right].$$

i.e. if b is very large compared with a_2 and a_1 we have $1/R \to 2ls_1\,a_1$ for all α, i.e. the total resistance is entirely due to the part under the cup.

(ii) *Large stomatal aperture.* When β_1 becomes very large ($\beta_2 \neq 0$) the limiting value is similarly found to be

$$m/R \to \frac{2ls_1}{s_1+s_2}\,a_1\,ms_1, \quad \text{or} \quad R \to \frac{1}{2la_1\,s_1}+\frac{1}{2la_1\,s_2},$$

i.e. the resistance is wholly due to the resistances of upper and lower epidermis under the cup. In the case of α (and β_2) $= 0$ the limiting value is given from (13) by

$$1/R = \frac{2l}{m}\left[\frac{\beta_1}{2+\beta_1(a_2-a_1)}\right],$$

i.e.
$$= \frac{2l}{m(a_2-a_1)}; \qquad m/R = \frac{2l}{a_2-a_1}. \quad (14)$$

The resistance here is entirely due to the two parts of the mesophyll under the washer, the resistances being in parallel.

Plotting $\log ms_1$ against $\log m/R$ for various values of α gives a series of curves similar to that of Fig. 11.

Determination of mesophyll resistance. An increase in the width of the washer, in practice the addition of the outer leaf chamber, will, under hypostomatous

conditions, lead to an expression similar to equation (13) which, inverted, we may write:

$$R' = \frac{m}{2l'\beta_1}\left[\coth\beta_1 a_1 + \coth\beta_1(b-a_3) + \beta_1(a_3-a_1)\right] \qquad (13')$$

$$R = \frac{m}{2l\beta_1}\left[\coth\beta_1 a_1 + \coth\beta_1(b-a_2) + \beta_1(a_2-a_1)\right]. \qquad (13)$$

As before, if the stomata are wide open, i.e. β_1 is large, a first approximation to m is given by

$$\frac{R'-R}{m} = \frac{a_3-a_1}{2l'} - \frac{a_2-a_1}{2l}, \qquad (15)$$

so that an approximate value of β_1 can be determined to be used in (13 and 13') to give a better value of m. If (15) is used without further correction the choice of a value for l' is important. End effects on the broad arc of the outer leaf chamber cannot be neglected, and some measure of compensation will be achieved by taking l' as the mean of l and the value obtained from the area of the outer chamber divided by its width ($2a_3$). The constants $a_3-a_1 = 0.8$, $a_2-a_1 = 0.2$, $l = 9.0$, $l' = \frac{1}{2}(9.0+10.6)$, lead to $(R'-R)/m = 0.030$, which is sufficient to give a rough value of m.

III. *Some general considerations.*

(i) *Units.* Physically, the 'resistance' of a system is the ratio of the driving potential difference to the current it produces; the preceding analysis has been based on this conception. Actually, however, the interrelations of R, m, and s_1 are such that any unit of resistance can be used. The usual resistance to fluid flow arises partly from the nature of the fluid itself and partly from the geometry of the system through which it flows, and the analysis will apply equally well to this latter part of the resistance. In this case a value of s_1 will represent $\sum_{n}\frac{r^4}{l}$, where n is the number of stomata per unit area and r and l are the radius and length of the equivalent capillary tube which would have the same resistance.

(ii) *Resistance across the leaf and m.* Neglect of the former seems reasonable in view of the extreme thinness of the leaf. If m is precisely known a check on this assumption is possible by measuring first the value of R_a under amphistomatous conditions and then sealing the upper surface to obtain R_h under hypostomatous conditions. If the resistance across the leaf *is* negligible the two values of ms_1 ought to agree. As we shall see below, the range of m/R in which this experiment could be usefully employed is one in which the accuracy of m is important and it might be more effective to use the experiment to determine m, assuming the resistance across the leaf is negligible. From the theoretical curves a derived curve of R_h/R_a against m/R_a can be plotted; from

the experiment a value of R_h/R_a is found, hence a value of m/R_a, which with the known value of R_a leads to a value of m.

The importance of m can be shown as follows. We have, at any point on the curves of Fig. 11,

$$\frac{\Delta \log ms_1}{\Delta \log m/R} = \mu \quad \text{say,}$$

from which, ignoring the constant factor which appears in both sides of the equation, we obtain

$$\frac{1}{ms_1}[s_1 \Delta m + m \Delta s_1] = \mu \frac{\Delta m}{m},$$

or
$$\Delta s_1/s_1 = (\mu - 1)\Delta m/m.$$

Thus when the slope is unity or near it the value of m is not very critical, but as μ approaches 2 the accuracy of m must be equal to the desired accuracy in s_1. For $\mu > 2$ the accuracy of m becomes even more important and with the approximate treatment necessarily involved in discussing the leaf chambers it is doubtful whether results from such parts of the curve ought to be used without some independent confirmation of the value of m.

(iii) *Limitations of the analysis.* The discussion of the leaf chambers is not as precise as that of the circular cup. For the narrow inner chamber the error is probably of the order of a few per cent.; this is an opinion and not a reasoned judgement. For the outer chamber, where the ratio of length to width is much smaller, one can only hope that the various sources of error do not all act in the same sense and that the final error is not serious. As this outer chamber has been used in the determination of m, every possible check on the result should be employed. The basic trouble is that experimental requirements and ease of analysis are mutually conflicting and a compromise must be found between obtaining results which cannot be interpreted and obtaining no results which can be interpreted with precision!

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Studies in the Inheritance of Physiological Characters

VI. Hybrid Vigour in the Tomato

Pt. IV. The Effect of Flower Removal on the Manifestation of Hybrid Vigour[1]

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INTRODUCTION

IN the preceding paper of this series (Hatcher, 1940) a study was made of the growth of two reciprocal tomato hybrids and their pure line parents, both the latter being varieties of *Lycopersicum esculentum*. Previous experiments (Ashby, 1937; Luckwill, 1937) had suggested that the manifestation of hybrid vigour in such hybrids was directly related to an initial advantage in embryo size: the author's investigation failed to confirm this hypothesis. Heterosis was noted in the hybrid embryo during development, but ultimately its effect was masked by the limitation set by the maternal environment, so that the size of the mature embryo reflected not its own genetical constitution but the fruiting characters of the maternal parent. The transient heterosis occurring during growth of the hybrid embryo was attributed to an inherent physiological vigour.

The hybrid plants did not again manifest heterosis until after the simultaneous onset of lateral formation and flowering, except in the cotyledons which in effect constituted the termination of embryo growth. When the plants were finally sampled considerable weight heterosis was apparent, and this was related to the inheritance of favourable characters from both parent varieties. Of the seven characters in which the parents differed three were mainly concerned—vegetative type, inflorescence type, and fruit size. The tall vegetative habit of one parent was associated with the production of large fruits, and a physiological consequence of this was a relatively small amount of lateral development; in contrast the dwarf vegetative habit of the other parent with its very small fruits was associated with a considerable degree of lateral development. In the proportions of total weight comprising fruits and laterals the hybrids were intermediate, though total weight itself was *not* intermediate but in excess of either parent, this excess representing the degree of hybrid

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[1] This work has been carried out while the author held a Beit Scientific Research Fellowship.

vigour. Hybrid vigour resulted, it seemed, from the combined effect of the different vegetative and fruiting characters of the parents, yet from the evidence available it could not be definitely stated whether these genetical factors were wholly responsible for the vigour of the mature hybrid plants, or whether some inherent physiological vigour as postulated in the case of the embryos was again the primary cause.

To attempt to elucidate this question it was decided to investigate the effect of removing flowers and so preventing the formation of fruits, thus eliminating the differential fruiting habits of the strains, although failing in the strictest sense to maintain the plants in a purely vegetative condition. Only the process of flower formation, however, is allowed to occur, and in this respect the habit of the tall parent is inherited as a dominant after a slight initial difference in the number of leaves before the first inflorescence.

The results of an experiment of removing flowers are herewith described as contributing to the analysis of hybrid vigour.

DESCRIPTION OF THE EXPERIMENT

The experiment was carried out during 1940, using the same parent varieties as in the earlier work (Hatcher, 1940), but details of these strains are again summarized here:

Parent varieties of *Lycopersicum esculentum*:

77—'Blaby', a tall plant with large red fruits, simple inflorescences, and homozygous for the factors D P O R Y S a h.

55—a dwarf plant with small yellow fruits, compound inflorescences, and homozygous for d p o r y s A h.

Reciprocal hybrids derived from these parents:

75 and 57—tall plants with medium-sized red fruits, simple inflorescences, and with the genetical constitution Dd Pp Oo Rr Yy Ss aA hh.

All these factors are described by Macarthur (1934).

Seeds were selected for size so that those of the hybrids approximated in weight to those of the maternal parent, with mean weights: 77, 3·492 mg.; 75, 3·559 mg.; 55, 1·917 mg.; 57, 2·042 mg. On March 21 seeds were sown in soil, two in a pot, and thinning to one seedling per pot two weeks from sowing gave twelve plants of each type. Five weeks from sowing plants were removed to the greenhouse where the experiment was to be completed, and a week later the eight most uniform of each type were selected for transplanting to 12-inch pots, in a mixture of loam, stable manure, and sand in the ratio of 3:1:1. Of the eight plants of each strain four were selected at random for flower removal, leaving four as control. The pots 2 ft. apart were arranged in two rows running along one side of the greenhouse and separated by a wide path. Allocation to row, and position within the row, was also at random.

Once flowering had started inflorescences were removed at weekly intervals from the plants selected for treatment. The effect was soon noticeable, but

no quantitative observations were made until the end of the experiment, twenty weeks from sowing.

PRESENTATION OF RESULTS

In Table I the main results are summarized, and the vertical columns in this table have been subjected to the analysis of variance. Values of 'F' (Variance/Error) are given in Table II, those which are significant being in italics, while in Table III treatment means with their significant differences are given.

TABLE I

Summary of Observed Data

Weight in kg. and height in cm. The abbreviations used are: Wt., weight; Ht., height; Tl., total; Mn., main shoot; Lat., lateral shoots; Fr., fruits; I., number of inflorescences on main shoot; L., number of leaves on main shoot.

Strain.	Row.	Treated plants.							Control plants.						
		Wt. Tl.	Wt. Mn.	Wt. Lat.	Wt. Fr.	I.	L.	Ht.	Wt. Tl.	Wt. Mn.	Wt. Lat.	Wt. Fr.	I.	L.	Ht.
77	1	1·71	0·34	1·37	—	9	34	144	2·02	0·23	0·38	1·41	8	32	96
		1·64	0·38	1·26	—	8	32	154	2·36	0·21	0·41	1·74	7	29	95
	2	2·13	0·43	1·70	—	9	36	142	2·61	0·27	0·57	1·77	8	32	112
		2·30	0·47	1·83	—	9	38	158	2·70	0·26	0·57	1·87	8	32	122
75	1	2·00	0·47	1·53	—	9	37	174	2·52	0·28	0·81	1·43	7	29	126
		1·69	0·41	1·28	—	9	36	153	3·70	0·40	1·02	2·28	8	36	157
	2	2·92	0·54	2·38	—	10	40	204	3·96	0·41	1·02	2·53	9	36	175
		2·77	0·52	2·25	—	10	39	198	2·70	0·30	0·50	1·90	8	33	110
57	1	2·14	0·45	1·69	—	9	39	171	3·65	0·38	0·91	2·36	9	37	146
		2·07	0·47	1·60	—	10	37	173	2·66	0·28	0·74	1·64	8	32	148
	2	3·00	0·51	2·49	—	9	37	172	3·45	0·33	1·05	2·07	8	32	125
		3·91	0·68	3·23	—	10	38	210	3·29	0·38	1·03	1·88	8	33	146
55	1	1·44	0·41	1·03	—	7	39	130	1·82	0·47	0·74	0·61	7	38	149
		1·69	0·43	1·26	—	8	40	135	1·78	0·43	0·61	0·74	8	39	147
	2	1·37	0·45	0·92	—	8	39	149	2·08	0·48	0·85	0·75	8	37	162
		1·17	0·38	0·79	—	7	40	139	2·15	0·40	0·75	1·00	6	34	116

TABLE II

'F' Values obtained by Analysis of Variance

Source of variance.		d.f.	Variate analysed.						Sig. F.	
			Wt. Tl.	Wt. Mn.	Wt. L.	I.	L.	Ht.	5%	1%
Strain	S	3	*17·72*	*7·59*	*24·82*	*8·48*	*9·68*	*7·24*	3·24	5·29
Treatment	T	1	*17·28*	*36·07*	*194·12*	*18·29*	*30·00*	*22·94*	4·49	8·53
Row	R	1	*11·11*	*6·38*	*25·31*	1·14	0·83	2·06	4·49	8·53
S×T		3	0·44	*6·45*	*11·99*	1·33	0·09	*3·54*	3·24	5·29
S×R		3	1·35	1·03	*6·19*	1·71	2·20	0·43	3·24	5·29
T×R		1	1·46	2·41	*10·64*	0·29	3·10	1·83	4·49	8·53
S×T×R		3	1·81	0·14	*7·18*	0·01	0·44	1·10	3·24	8·53
Error		16	—	—	—	—	—	—	—	—

Significant 'F' values in italics. For abbreviations used see Table I.

TABLE III

The Effect of Treatment on Strain

Weight in kg. and height in cm.   T = treated; C = control.

Strain.	Total weight.		Weight of main shoot.		Weight of laterals.		No. of inflor.		No. of leaves.		Height.	
	T	C	T	C	T	C	T	C	T	C	T	C
77	1·945	2·422	0·405	0·242	1·540	0·482	8·7	7·7	35·0	31·0	149	106
75	2·345	3·220	0·485	0·347	1·860	0·837	9·5	8·0	38·0	33·5	182	142
57	2·780	3·262	0·527	0·342	2·250	0·932	9·5	8·2	37·7	33·5	181	141
55	1·417	1·957	0·417	0·445	1·000	0·737	7·5	7·2	39·5	37·0	138	143
Sig. diff.	0·606		0·081		0·278		0·97		2·9		26	

Total fresh weight.

The plants from which flowers were removed were of lower weight than the controls in all the strains. The strain-treatment interaction variance was not significant, thus the treated and control hybrid plants manifested hybrid vigour to the same degree. If heterosis is expressed as the percentage of the mean hybrid weight over that of the heavier parent, then the value for treated plants without fruits is 32 per cent. while the control plants allowed to set fruits give a value of 34 per cent. There is thus evidence of equal heterosis with and without fruit formation.

Weight of main shoot.

As the significant interaction of strain and treatment shows, the effect of removing flowers was not uniform throughout the genotypes. In parent 77 and the hybrids a significant increase in main stem weight was noted, but not so in parent 55. In the treated plants there was no difference in main stem weight between the two parent types; the hybrids, however, showed heterosis. In contrast, where fruits developed, the greatest difference in stem weight was found between the parent types, the hybrids being intermediate. Thus in the presence of fruits there was no heterosis shown in main stem growth, though when fruiting was suppressed heterosis was shown.

Weight of lateral shoots.

Table II shows that in this character the effects of the factors were more marked than in any other, all the main and interaction effects being highly significant. In many respects the results resemble those for main shoot weight: the control hybrid plants did not manifest heterosis, while, as a result of fruit suppression, differential genotype increases in lateral weight—not significant in the case of parent 55—led to the manifestation of heterosis in the treated plants.

Number of inflorescences and leaves on main shoot.

The only significant variances were due to strain and treatment, and the

effect of the latter was to increase inflorescence and leaf number in all the strains. The parent 55 produced fewer inflorescences than the other strains which did not differ significantly among themselves, though the figures suggest a higher number in the hybrids. The parent 77 produced the fewest leaves, but whereas in the treated plants the other strains did not differ markedly, in the controls the parent 55 produced significantly more leaves than the hybrids. Leaf number thus displayed heterosis either in treated or control plants.

Height of main shoot.

Treatment increased the height of parent 77 and the two hybrids, but did not affect the height of the parent 55, this result resembling that for main shoot weight (Table III). The control hybrids did not manifest heterosis over the parent 55, but the treated hybrids were much taller than either parent. It should be pointed out that in the years 1938 and 1939 height heterosis was present in the normally fruiting plants.

The heterosis relations have been summarized in Table IV, in which the degree of heterosis, expressed as percentage excess of the hybrids over the larger parent, are given.

TABLE IV

Heterosis (%) of Treated and Control Plants

With fruit (Control).	%	Without fruit (Treated).	%
Total weight	34	Total weight	32
Lateral weight	20*	Main shoot weight	20
Inflorescence no.	5*	Lateral weight	34
		Inflorescence no.	9*
		Height	22
No heterosis in		No heterosis in	
Leaf no.		Leaf no.	
Height			
Main shoot weight			

* Not significant.

Fruiting relations in control plants.

The results of the analysis of variance are presented in Table V*a* and the distribution of fruit on the plant for the various strains in Table V*b*. Heterosis in total fruit weight amounted to 18 per cent., and this was due entirely to the effect of the lateral shoots, no heterosis being manifest in the fruit on the main shoot.

TABLE V

Fruit Weights of Control Plants

(a) '*F*' *Values from Variance Analysis* (italicized if significant).

Source of variance.		Total wt. fruits.	Variate analysed. Wt. fruits on main shoot.	Wt. fruits on laterals.	Sig. F. 5%	1%
Strain	S	*11·71*	4·79	*17·99*	4·07	7·59
Row	R	1·30	3·63	0·37	5·32	11·26
S × R		0·22	0·61	1·92	4·07	7·59

(b) *The Relation of Strain to Weight (kg.) of Fruit.*

Strain.	Total wt. fruits.	Wt. fruits on main shoot.	Wt. fruits on laterals.
77	1·70	1·28	0·42
75	2·03	1·21	0·82
57	1·99	1·20	0·79
55	0·77	0·67	0·10
Sig. diff.	0·56	0·42	0·26

The effect of row.

Variance due to row was significant in the analyses on total weight, stem weight, and lateral weight (Table II). The higher values were associated with row 2, the one nearer the side of the greenhouse, and it is probable that the operating factor was light intensity. In the control plants fruit weight was not affected by this factor. Significant interaction variances involving row were found only in the analysis of lateral weight where even the triple interaction of strain, treatment, and row was highly significant. In Table VI row means are given and the following points are to be noticed:

TABLE VI

Weight of Lateral Shoots as influenced by Row

	Strain.	77.	75.	57.	55.
Treated	Row 1	1·31	1·40	1·64	1·14
	Row 2	1·76	2·31	2·86	0·85
Control	Row 1	0·39	0·91	0·82	0·67
	Row 2	0·57	0.76	1·04	0·80
Sig. diff.			0·39		

(a) Weight of lateral shoots was in every case significantly greater in the treated plants compared with their controls except parent 55 in row 2. (b) Control plants did not differ significantly in lateral weight between the rows. (c) In the treated plants only parent 55 showed no row effect. (d) The difference of lateral weight between rows was considerably greater in the hybrids than in the parent 77. (e) Even reciprocal hybrids differed significantly in lateral weight in row 2, though this was the only instance throughout the analyses where such a significant difference was found.

DISCUSSION

Physiological studies of hybrid vigour, while suggesting that it may be present in all stages of the life-history, also show conclusively that in any given hybrid its manifestation may be restricted to particular periods of development. The tomato hybrids 75 and 57 provided an example of this (Hatcher, 1940), for though they showed heterosis during embryo development and after the onset of flowering, the mature embryo was the same size as the maternal parent, while the young vegetative plants completely resembled the parent 77. This embryo heterosis was attributed to an in-

herent physiological vigour, but the heterosis of the mature plants was related to definite genetical factors controlling fruiting and vegetative growth. The present experiment on the effect of removing flowers was an attempt to establish evidence of a relationship between the heterosis of the embryo and of the mature plant.

Comparison of total weights of treated and control plants (Table III) shows that when fruiting is artificially prevented the extra vegetative growth resulting fails to compensate in weight for the absent fruit. If, however, the vegetative parts are alone considered the weights attained in the treated and control plants are as shown below in Table VII. The increase in vegetative growth

TABLE VII

Weights (kg.) of Vegetative Parts

Strain.	Treated.			Control.		
	Main shoot.	Laterals.	Total.	Main shoot.	Laterals.	Total.
77	0·41	1·54	1·95	0·24	0·48	0·72
75	0·49	1·86	2·35	0·35	0·84	1·19
57	0·53	2·25	2·78	0·34	0·93	1·27
55	0·42	1·00	1·42	0·45	0·74	1·19

due to fruit suppression differs greatly in the different strains; in parent 77, 170 per cent.; hybrid 75, 97 per cent.; hybrid 57, 118 per cent.; parent 55, 18 per cent. It is evident, therefore, that the limitation impressed on vegetative development varies considerably, and is very much greater in the parent 77 than in the hybrids, and in these again than in the parent 55. It should be noted that so far as vegetative growth is concerned there is no heterosis to be observed in the control plants, the hybrids resembling parent 55, and all strains being greater than parent 77. When, however, the limitation in growth imposed by fruiting is removed, it is seen that now (1) the hybrids display heterosis in vegetative growth, and (2) parent 77 exceeds parent 55. Yet in spite of the fact that vegetative growth in the control plants shows no heterosis effect, the marked development of lateral shoots in the hybrids compared with parent 77 is of some consequence in considering the heterosis effect on fruit production. Referring to Table V*b* it is seen that in so far as fruit on the main stem is concerned no heterosis appears, the fruit weight of the hybrids being the same as that of parent 77 (hybrids 1·21 kg., parent 77 1·28 kg.), but with respect to fruit borne on lateral shoots very great heterosis is displayed (hybrids 0·81 kg., parent 77 0·42 kg.). The major heterosis effect on total fruit production is thus to be attributed to the more vigorous lateral development in the hybrids compared with parent 77, though in vegetative growth itself the hybrids do not manifest hybrid vigour but are quantitatively equivalent to parent 55. It is probable that such equivalence is fortuitous, for the greater fruit size of the hybrids, and the consequently greater check impressed on vegetative growth, masks an inherently greater capacity for growth of this

type compared with the dwarf parent. This is no doubt the effect of the factor D inherited dominantly from parent 77, though in this parent vegetative growth is suppressed by fruiting to an even greater degree. The combined effects of vegetative and fruiting characters inherited from the parent types are thus intimately concerned in the manifestation of, but do not in fact satisfactorily account for, the hybrid vigour seen in the heterozygous forms, since when fruiting limitations are removed the hybrids still display heterosis to the same extent. Some specific effect of hybridity leading to greater vigour must therefore be postulated for the whole plant as has already been suggested for the embryo.

SUMMARY

A further experiment with two varieties of *Lycopersicum esculentum* and their reciprocal hybrids is described. The effect of removing flowers as they formed, thus preventing fruit development, was to decrease the final weights of the plants but not to interfere with the manifestation of hybrid vigour. It is concluded that the combination in the hybrids of vegetative and fruiting characters inherited from the parents does not of itself account for heterosis. It is postulated for the whole plant, as was previously postulated for the embryo, that some specific effect of hybridity leads to the greater vigour of growth in the hybrids.

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Studies in Growth Analysis of the Cotton Plant under Irrigation in the Sudan

II. Seasonal Variation in Development and Yield

BY

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With six Figures in the Text

INTRODUCTION

THIS paper discusses an experiment carried out in the Sudan Gezira Irrigation Scheme, initiated by A. R. Lambert to record seasonal observations on the growth of the cotton crop. By season 1925–6, with the opening of the Sennar Dam, the scheme was in full operation, and as early as 1927–8 the observation plot was established at the Gezira Research Farm. Subsequently for fourteen years all agricultural operations on the plot have kept closely to a predetermined schedule and growth records have been systematically collected. The experiment is still in progress.

These records provide extensive material for a study of seasonal variation in crop development, and thus it has become possible not only to assess in advance the prospects of a single crop but also to throw light on the origin of annual yield fluctuations in the Gezira. The practical value of a study of seasonal influences is evident, for when the operative seasonal factors have been identified agricultural practice can be adapted to counteract adverse effects and thus improve final yield.

Standardization of agricultural practices, of soil and plant variety, is essential for reliable comparison of crop development in successive seasons; otherwise environmental influences on the crop are inseparably mingled with irregularities from other sources.

Very early in its history the experiment began to prove its worth, for since there were some very low yields in the Gezira Scheme in the period 1930–1 to 1933–4, there are data available from lean years as well as from those of higher yield, thus giving critical information on the stage of development within any season at which the environment became especially unfavourable and of the nature of the resulting interference with growth.

DESCRIPTION OF FIELD LAYOUT AND OBSERVATIONS

The experiment has been described in detail by Lambert in the annual reports of the Agricultural Research Institute, Sudan (Lambert, 1928–38). The agricultural details may be summarized as follows:

Rotation. Three-year. (i) Cotton; (ii) *Dolichos lablab*; (iii) Fallow.

Cotton. Variety.   Sakellaridis (*Gossypium barbadense*), 'Massey's Selected Domains' Strain.

Sowing date. August 19.

Spacing.   80 cm. between ridges, 50 cm. between holes; thinned at 4 and 6 weeks, finally to 2 plants per hole.

Manuring.   300 rotls[1] ammonium sulphate per feddan,[1] applied 6 weeks after sowing.

Harvest.   First picking late December, then fortnightly to last picking late April.

The gross area of the experiment is $3\frac{1}{2}$ feddans and comprises six blocks, two of each phase of the rotation, the position of the phases having been determined by randomizing at the start of the experiment. The blocks are surrounded by a belt of *Cajanus indicus*, sown afresh each year, to minimize humidity changes from the varying proximity of adjacent cultivation, and to check the entry of blackarm (*B. malvacearum*) disease.

The observations made on the crop up to the time of harvest include (*a*) field records of height, flowers, and bolls, (*b*) measures of pest and disease damage, and (*c*) plant samples for dry matter and nitrogen determinations. Table I gives the frequency of the observations on growth and the number of plants examined. With pest and disease damage, the extent of observations varied according to the extent of the damage. To facilitate the taking of observations each block was divided into quarters and these again split into thirds, one for yield and field observations, one reserved for dry matter sampling, for here the stand suffered by removal of plants, and one for soil examination. The Soil Research Section, A.R.I., has conducted regular soil nitrate analyses since 1930, the results of which have been published by Greene (1939).

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TABLE I

Type and Frequency of Growth Observations

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Observations.	No. of plants examined and frequency of examination.	No. of seasons for which data available.	Remarks.
1. Height, main stem	100 every 14 days	13	Same random sample
2. No. of Nodes, main stem	,, ,,	11	observed through-
3. Defoliation, main stem	,, ,,	11	out season.
4. No. of Flowers	200 daily	13	
5. Leaves, dry wt.	80 every 14 days	10	10 plants at random
6. Stem, dry wt.	,, ,,	10	in each quarter
7. Tops, dry wt.	,, ,,	10	block.
8. Nitrogen in leaf (per cent. of dry matter)	,, ,,	10	Plants, used for dry weights, from a
9. Total nitrogen-tops	,, ,,	10	single block grouped.
10. Yield	200	13	Same plants as for flower count.

[1] 1 rotl = 0·99 lb. = 450 gm.; 1 kantar = 315 rotls; 1 feddan = 1·038 acres.

For some observations data are available from more seasons than for others. The method and details of observational technique have already been outlined (Crowther, F., 1934), and call for no further comment.

Primary Data of Separate Seasons

Most of the primary data are summarized in Figs. 1–3, and the cumulative yields at harvest are also shown. In all cases records are expressed on the basis of a single plant.

Since this paper concerns cotton yields as determined by seasonal influences exerted at the various stages of the plant's development, the scope is restricted to a study of the relation of the developmental data to the yield. Such aspects as the change with time of the various characteristics studied and their interrelation are only mentioned where they elucidate the problem of seasonal yield variation.

From the data in Fig. 1 (*a*) (heights of main stem) it will be seen that seasonal differences in *height* are great in the later months, the plants from November onwards being as much as 50 per cent. taller in some seasons than in others. In general plants have been taller in recent years. *Nodes*, recording the numbers of leaves (nodes of main stem, Fig. 1 (*b*)), show smaller divergencies between seasons. This contrast in variability is emphasized in Table II, which gives the standard deviation for a single season of each of the variates listed in Table I.

Table II

Standard Deviation of a Single Season for Growth Observations (percentage of appropriate monthly mean)

	Aug.	Sept.	Oct.	Nov.	Dec.
1. Height	12·8	13·4	18·9	19·1	18·0
2. No. of Nodes	4·70	5·82	7·25	4·63	4·51
3. Internode length . . .	10·9	15·5	14·9	17·9	19·3
4. Defoliation	—	—	31·8	24·9	*
5. No. of Flowers	—	—	42·1	33·3	34·9
6. Leaf, dry wt.	24·2	22·0	29·4	46·4	51·8
7. Stem, dry wt.	20·4	26·2	35·1	46·3	51·2
8. Tops, dry wt.	22·0	23·2	32·3	46·6	50·4
9. Leaf nitrogen	10·5	4·9	3·8	6·5	7·3
10. Total nitrogen, tops . . .	17·9	28·1	38·8	50·4	50·3
11. Final yield			48·1		

Standard deviation for height is from two to four times as great as that for nodes, depending upon the stage of crop development. Consequently height differences must primarily reflect differences in internode extension. Whereas the standard deviation for internode length increases progressively month after month, that for node number reaches a maximum in mid-season, i.e. October. As a result height differences are proportionally as great then as in any subsequent month. The small difference in node number for December

* No data available here or in Tables III and IV for defoliation in December.
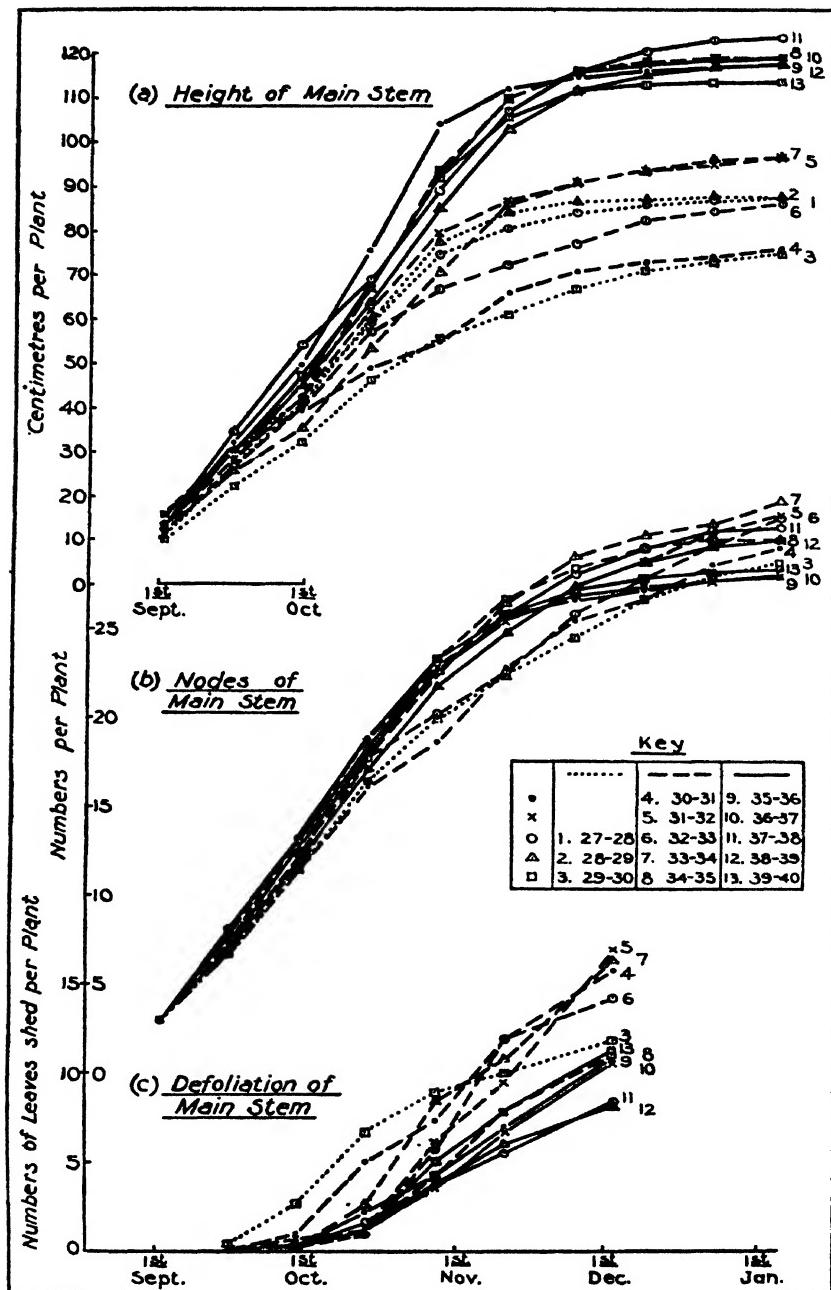
FIG. 1. Graphs showing (*a*) heights, (*b*) nodes, and (*c*) defoliation, of main stem for separate seasons. The key refers to Figs. 1 to 4.

from year to year probably arises from the controlling influence of 'internal starvation' (Gregory, 1928). A small plant ripens earlier than a large one, since the extra yield of the latter is produced at later-formed nodes. In years of small growth node production is checked earlier and is *resumed earlier* than in a year of more vigorous growth. This resumption with small growth, occurring in December, coincides with the period of slackening of node production in more vigorous plants.

Though the numbers of leaves eventually produced on the main stem are fairly constant, the persistence of the leaves varies considerably. *Defoliation* (defoliation of main stem, Fig. 1c) begins in late September, about thinning time, with the shedding of the cotyledons, and spreads from the lower leaves upwards. During October it may increase rapidly, causing the loss of half the main-stem leaves produced; on the other hand, in some years the loss in October is slight. Defoliation, increases progressively in later months and frequently by the end of December the plant is left bare in the middle and lower portions.

Mid-October regularly marks the onset of *flowering* (Fig. 2a) but thereafter the rate of flowering and the total numbers produced vary greatly from season to season, by as much as threefold. This seasonal variability is reflected in the standard deviation for flowers in Table II, larger at all times than that for either height, defoliation, or node number. Recent years have produced plants not only taller but bearing a greater number of flowers.

Turning to observations of dry matter, *leaf weights* (Fig. 2b) vary considerably from year to year, in fact leaf and stem dry matter, and the dry matter of the whole tops, all of which have similar standard deviations, are consistently more variable than all other growth observations, and are of a magnitude similar to that of the final yield itself (see Table II). The date of maximum leaf weight varies from year to year, and in some years a clearly defined maximum appears; all years have low values in March. *Stem weights* (Fig. 2c.), which include branches and petioles, by contrast, mostly continue to increase until the crop is uprooted, for shedding represents only a small loss of dry matter. The *total dry matter of the tops* is not graphed. It follows a curve similar to that of stem weight in the early months and reaches a maximum in late January before the onset of severe fruit shedding. Before this, seasonal differences resemble those for leaf and stem but later the influence of fruiting is predominant.

Analyses of dry matter show that seasons differ markedly in the percentage content of *leaf nitrogen* (Fig. 3a). *The earliest determinations, made within a day or two of germination and only seven days after the crop is sown, have the largest standard deviation per season as well as the highest values for nitrogen content.* The mean percentage at the end of August, a fortnight after sowing, is 4·75 per cent., varying with seasons between 3·85 per cent. and 5·44 per cent., a range of 1·59, compared with 0·50 for December. During September the leaf-nitrogen content fluctuates erratically through the influence of external
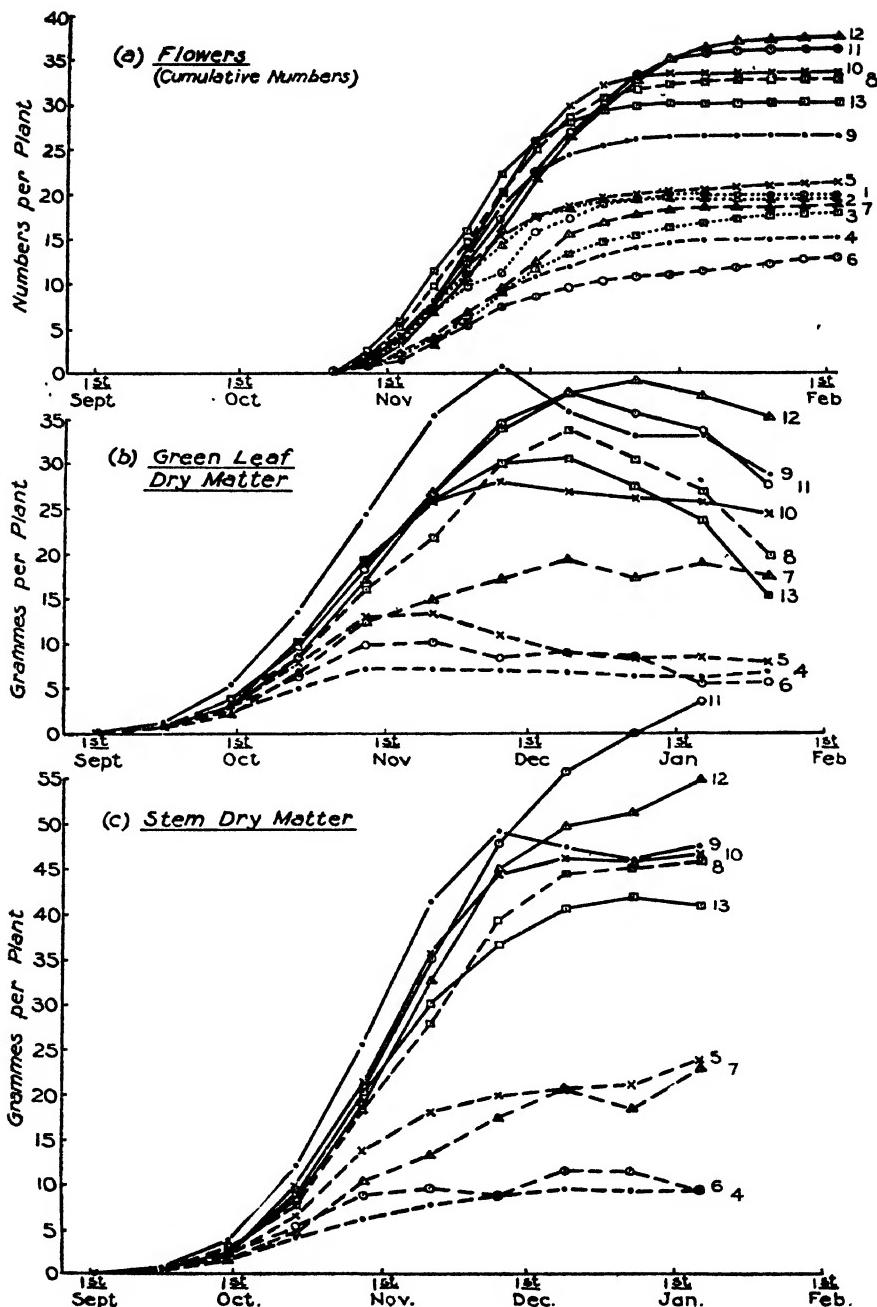
Fig. 2. Graphs showing (*a*) flower number, (*b*) green leaf dry weight, and (*c*) stem dry weights for separate seasons. For key see Fig. 1.
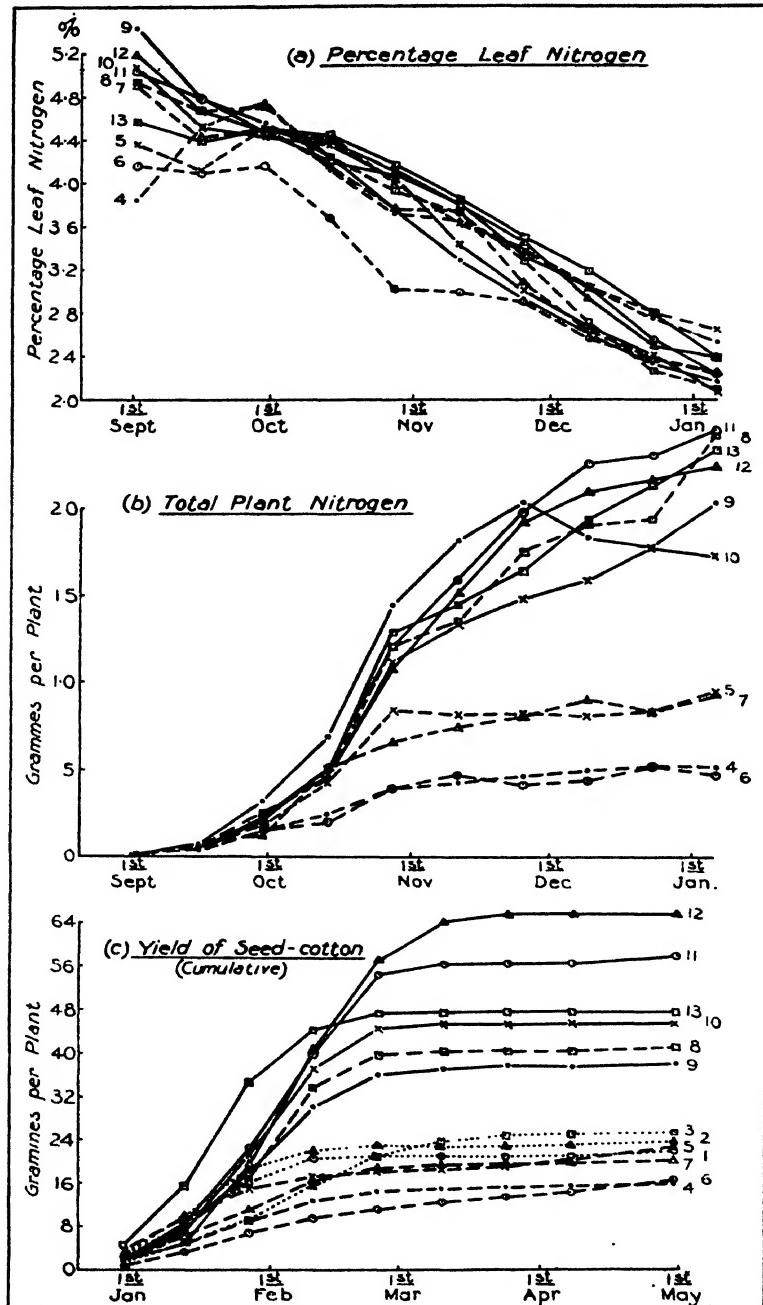
FIG. 3. Graphs showing (*a*) percentage leaf nitrogen, (*b*) total plant nitrogen, and (*c*) yield of seed cotton for separate seasons. For key see Fig. 1.

factors, but at the end of the month the percentage varies little from year to year, and this also applies to succeeding months. Analyses of stem nitrogen show a progressive fall from germination onwards and as in leaves the discrepancies between seasons are greatest in the earliest determinations. Flower-bud analyses in contrast with both leaves and stems, show seasonal differences *increasing* progressively during development. Thus the lowest and highest values in January for the separate seasons are 2·2 per cent. and 3·5 per cent. respectively. The data for stem and flower bud are not given further in this paper but they have been used to calculate the *total nitrogen in the tops* (Fig. 3*b*). Here the changes with time from October to December closely follow the corresponding curves for total dry matter, for the plants in this period consist mainly of leaf and stem, neither of which as already stated show pronounced seasonal variation in percentage nitrogen. After December the curves are modified by the increasing influence of fruiting, at final harvest about half the total nitrogen in the tops usually being accumulated in the fruits.

The cotton crop is harvested in fortnightly pickings of seed cotton (see *c* plus lint), and the cumulative picking weights are given in Fig. 3 (*c*) for comparison with the growth curves. The bolls open from mid-December onwards and the first picking is usually gathered at the end of December, although the crop varies considerably from year to year in the stage of maturity at this time. Pickings are heaviest during January but seasonal differences are greatest in February, years of small yield producing little at this time while those of large yield may crop as heavily then as in January. Final yields vary greatly from year to year (see Table II), being in some years four times that of others. The yields fall into two groups, those after 1934 being without exception greater than those of earlier years. Thus there has been a very marked improvement in the yield of the experiment over the run of years covered by these observations.

REGRESSIONS OF YIELD ON DEVELOPMENTAL VARIATES

The curves for yield described above correspond roughly to those of height, stem-weight, and other cumulative curves, except, of course, for a shift along the time scale. With the exception of defoliation all the curves show maximum values in recent years, indicating a general positive correlation between development and yield, at least in later months. For defoliation recent years have mostly shown low values, suggesting the existence of a negative correlation between amount of leaf-shedding and yield. Comparison of seasonal differences in development by this graphical method provides only a demonstration of the major differences within any one set of observations and a rough determination of the order for the seasons of the values of the characteristics studied and of yield. Recourse is necessary to statistical methods to assess more accurately the degree of correspondence between development and yield.

The regressions of the final yields on the various growth characteristics,

each considered separately, are presented in Table III. The regressions have been calculated month by month up to the beginning of crop harvest at the end of December, from the data presented in Figs. 1 to 3 using the same units. The values used refer to the end of the month except those for percentage nitrogen, which after August are the means for the whole month. All available data are utilized so that some regressions are based on 13 years' and others on 10 years' data.

TABLE III

Yield Regressions on Monthly Developmental Data

		Yield regressions.					Standard errors of regressions.			
	Aug.	Sept.	Oct.	Nov.	Dec.	Aug.	Sept.	Oct.	Nov.	Dec.
1. Height .	+3·03	+2·01	+0·74	+0·74	+0·77	2·82	0·61	0·24	0·14	0·14
2. No. of nodes	+9·21	+10·43	+5·53	+4·69	−2·30	40·99	7·08	3·10	4·19	4·23
3. Internode length .	+9·8	+19·5	+22·6	+21·9	+21·0	11·32	6·55	7·40	4·98	4·83
4. Defoliation	—	−8·50	−7·22	−5·06	*	—	7·44	2·43	0·80	*
5. Flowers .	—	—	+12·60	+2·15	+1·84	—	—	7·04	0·49	0·40
6. Leaf, d. wt.	+128·0	+15·3	+3·69	+1·39	+1·32	136·5	17·7	1·57	0·34	0·20
7. Stem, d. wt.	+463·4	+29·7	+3·81	+1·10	+0·93	256·8	28·3	1·20	0·23	0·12
8. Total, d. wt.	+108·3	+11·1	+1·81	+0·56	+0·40	87·9	9·77	0·66	0·12	0·05
9. % Leaf N .	+24·7	+54·9	+64·8	+37·4	−3·9	9·1	20·1	32·4	24·6	32·6
10. Total N .	+152·8	+3·52	+0·90	+0·54	+0·49	73·8	2·54	0·27	0·10	0·09

With the exception of defoliation the regressions are positive, showing that yields increase with larger plants, size being measured either by height, dry weight, or flower production; but many of the regression coefficients in the early months are within the limits of the standard error and thus not significant.

From October onwards every cm. of height above the mean indicates an extra yield of 0·7 gm. per plant. Similarly one extra gramme of leaf dry matter in October is associated on an average with 3·7 gm. increase in final yield. In November or December on the other hand a similar increase in leaf weight implies an increased yield of only 1·3 gm. Each additional flower in November and December is associated with a yield increase of about 2 gm. which, as would be expected, closely tallies with the average weight of seed cotton produced per boll. Heavy defoliation in October and November is associated with drastic reduction in yield. Every extra leaf shed during this period indicates a lowering of yield below the mean by 5 to 7 gm. or a loss of two to three bolls per plant.

Most of the regression coefficients in Table III diminish with time because of the increase in the monthly mean values of the particular characteristic concerned, as seen in Table IV. Difficulty in comparing the magnitude of the regressions in successive months can be overcome by expressing them in terms of their appropriate standard errors by the 't' test, as given in the right-hand half of the table (IV). This has the twofold advantage of correcting for differences in means and measuring the significance of the monthly regressions on the same scale.

TABLE IV

Monthly Means of Developmental Data and 't' Values for Significance of Yield Regression Coefficients†

	Unit.	Monthly means.					't' Values of Regression Coefficients.				
		Aug.	Sept.	Oct.	Nov.	Dec.	Aug.	Sept.	Oct.	Nov.	Dec.
1. Height . .	cm. p.p.	13·1	42·8	79·7	97·9	100·6	1·08	3·29‖	3·04‖	5·17‖	5·36‖
2. Nodes . .	no. „	3·0	12·5	21·9	27·6	29·5	0·23	1·47	1·79	1·12	−0·54
3. Internode length .	cm.	4·5	4·0	3·6	3·6	3·5	0·87	2·98§	3·05§	4·41‖	4·34‖
4. Defoliation .	no. „	—	0·57	5·31	12·36	•	—	1·14	2·97§	6·31‖	•
5. Flowers .	„ „	—	—	1·5	18·3	24·1	—	—	1·79	4·37‖	9·78‖
6. Leaf, d. wt. .	gm. „	0·19	1·54	10·4	22·6	23·7	0·94	0·86	2·35‡	4·11‖	6·49‖
7. Stem, d. wt. .	„ „	0·10	1·03	9·9	28·3	35·0	1·81	1·05	3·17§	4·89‖	7·52‖
8. Total, d. wt. .	„ „	0·30	2·57	21·2	58·5	83·5	1·23	1·13	2·75‡	4·67‖	8·10‖
9. N (%) in Leaves .	% of d. wt.	4·75	4·55	4·20	3·46	2·70	2·71‡	2·73‡	2·00	1·52	−0·12
10. Total N, tops	gm. p.p.	0·01	0·20	0·97	1·44	1·61	2·07	1·39	3·33§	5·18‖	5·74‖

† Theoretical 't' values for 10 to 13 years. For P = 0·2, t = 1·36–1·40; ‡ for P = 0·05, t = 2·20–2·31; § for P = 0·02, t = 2·72–2·90; ‖ for P = 0·01, t = 3·11–3·36.

The theoretical 't' values for 10 and 13 years differ only slightly (see footnote) and Table IV therefore allows of comparison between different growth measures in the degree of correlation with final yield.

Height is closely correlated with yield from late September (the time when the crop is thinned), onwards until harvested. By contrast node numbers, which were shown in Table II to vary little from year to year, are not significantly correlated with yield at any stage. The height-yield association must therefore be based on seasonal differences in internode length, which is confirmed by the significance of internode length regressions from September onwards. The correlation of height with yield has already been used in the Sudan (Crowther, 1934) for predicting yield differences between treatments within a single experiment. It is evidently a most reliable as well as an easily obtained record for forecasting the Gezira crop early in the season. There is no gain in information by using internode length instead of height.

Cumulative flower-numbers are highly correlated with yield as soon as flowering is in full swing, i.e. from November onwards. This is to be expected, since yield is the product of fruit number and size, but it follows that boll shedding is evidently of secondary importance. Leaf, stem, and total dry matter regressions are all significant from October, which covers the whole growth period subsequent to thinning.

Dry matter and height records both measure vegetative vigour and therefore the September values provide an interesting contrast, for whereas height regression is significant that for dry matter is not. The difference can be explained on morphological grounds. The earliest internodes are free to expand just as are those formed later, and plant height is therefore sensitive to environmental influence from germination onwards. Dry matter production on the other hand, even under favourable conditions, is controlled by the rate of formation of new leaves and of their expansion, and therefore cannot increase beyond a maximum determined by morphological development, however favourable the external conditions. Some differences in dry matter

production as between seasons result from factors such as storm damage or flea-beetle attack, which may destroy the leaf surface, but these influences are only temporary and are quite incidental to yield determination.

Regression of yield on the percentage nitrogen content of the green leaves reaches significance as early as late August when the plants are not more than twelve days old. This correlation was first noted by Lambert (1928–38) in data for the five seasons 1932–3 to 1936–7. As most of the nitrogen in the seed is present in the cotyledons, possibly differences in the percentage leaf nitrogen soon after germination might arise from initial seed differences, but as will be shown later, the yields of this observation plot, sown with locally grown seed, varied from season to season in a manner similar to the adjacent commercial area, which was sown with seed from other parts of the Sudan or from Egypt. It is, therefore, unlikely that seed differences can be primarily responsible for the leaf-nitrogen correlation. More probably the correlation arises from seasonal differences in rate of nutrient absorption by the developing seedlings. The seedlings break through the soil surface four days after sowing and Lambert showed the correlation to be significant for samples collected only three days later. This is a remarkably short period in the development of a crop which has yet eight months to grow before harvest is complete. The first true leaf does not usually appear until eight days after sowing, so it is the nitrogen in the cotyledons which is primarily responsible for this early correlation with yield.

The regression of yield on leaf nitrogen percentage is also significant in September, but thereafter the correlation with yield declines, unlike all the other characteristics studied for which increase in significance is found as the season advances. Evidently, as dry matter accumulation is limited by the rate of morphological development, vigorous root activity may result in accumulation of nitrogen within the plant. Later the rapid increase in plant size entails an increased demand for nitrogen, but in the nitrogen-deficient Gezira soil the supply is frequently inadequate, so that the nitrogen concentration within the vegetative organs falls to such an extent that leaf growth is curtailed and the rate of dry matter production thus cut down (Crowther, 1934). The extent of interference with leaf growth will depend upon the degree of nitrogen deficiency within the plant, with the result that ultimately the early differences in leaf-nitrogen percentage are replaced by corresponding differences in amount of leaf and total dry matter.

By contrast with percentage leaf nitrogen, the total amount of nitrogen in the tops during August and September is no guide to prospective yield. In later months yield regressions on total nitrogen are similar in significance to those on total dry matter.

Thus the earliest index of yield is percentage leaf nitrogen in August; in September this is joined by plant height. In later months height, dry matter, and defoliation are all correlated with yield, and from November flower-numbers also.

These conclusions refer to comparisons of growth variates singly with yield. They do not show how far the information from one variate is supplementary to that from another and how far it is duplicated. Thus, for example, the relative order of the seasons for early leaf nitrogen may be strictly maintained later for plant height, dry matter, and defoliation; the relative order may nevertheless be modified during development despite each stage continuing to show correlation with final yield.

TABLE V

Amount of Yield Variance accounted for by Single and Partial Regressions

Single variate.	%	Two variates.	%	Three variates.	%
End August					
% N leaves	47·9*	% N leaves and height	50·0		
Height	3·7	% N leaves and stem dry wt.	50·6	% N leaves, height, and stem dry wt.	52·4
Stem dry wt.	29·0	Height and stem dry wt.	37·0		
End September					
% N leaves	48·2*	% N leaves and height	65·5		
Height	55·3*	% N leaves and stem dry wt.	50·0	% N leaves, height, and stem dry wt.	76·9
Stem dry wt.	20·6	Height and stem	67·6		
End October					
Height	46·8*	Height and stem dry wt.	56·5		
Stem dry wt.	55·6*	Height and defoliation	69·6	Height, stem dry wt., and defoliation	73·5
Defoliation	69·5†	Stem and defoliation	70·9		
End November					
Height	74·7†	Height and stem dry wt.	76·2	Height, stem dry wt., and flowers	76·6
Stem dry wt.	74·8†	Height and flower	74·8	Height, stem dry wt., and defoliation	89·7*
Defoliation	86·7†	Height and defoliation	89·3*	Height, flowers, and defoliation	89·8*
Flowers	63·9†	Stem dry wt. and flowers	75·1	Stem dry wt., flowers, and defoliation	89·9*
		Stem dry wt. and defoliation	87·9*		
		Flowers and defoliation	89·7†		
End December					
Height	76·0†	Height and stem	90·1*		
Stem dry wt.	87·6†	Height and flower	88·8*	Height, stem, and flowers	92·9
Flowers	88·7†	Stem and flowers	90·0		

* Indicates significance for the level $P < 0.05$ and † indicates that for the level $P < 0.01$.

For consideration of their simultaneous effects recourse is necessary to partial regression coefficients, given in Table V where, for ease in comparing the degree of association with final yield, the data are presented in the form of percentage of total yield variance accounted for by a particular set of partial regressions or by the simple regressions. Significance is denoted by the symbols given in the footnote of Table V. In the case of partial regressions, symbols are only included when the addition of a second or third variate has significantly increased the amount of yield variance accounted for. For

example in October, 'Height and defoliation' is significant compared with 'Height' alone, but not necessarily with 'Defoliation' alone. In calculating these regressions the data for all variates have of necessity been limited to the last ten seasons and are therefore slightly different from those on which Tables II to IV are based. Since ten years is a short period for calculating regressions, the number of variates considered simultaneously is limited to three. Not all the variates considered earlier and entered in Table III have been examined in combination. Thus node number and internode length have been excluded, the former because no relation is found with yield and the latter for its resemblance to plant height. The three measures of dry matter are highly intercorrelated, hence stem weight was selected as less affected by irregularities due to leaf shedding.

Examination of Table V shows a progressive increase from August to December in the degree of correlation between developmental records and yield. The efficiency of developmental data in measuring the extent of seasonal yield variation is amply demonstrated, for values as high as 90 per cent. show that factors which differentially affect yield and these developmental variates are incidental. For example, a value of 90 per cent. for December shows that seasonal differences in subsequent months, January to April, can play only a minor role. Since the December regressions include fruit counts, a fairly high degree of association with yield is to be expected, but with regard to the earlier months it is remarkable to find a value as high as 50 per cent. for August, i.e. within a fortnight of sowing. This August value arises entirely from the percentage leaf nitrogen correlation, for no other correlation even approaches significance at this time. This variate is not usually included in a programme of growth observations and correlation with very early growth may therefore be overlooked.

At thinning time, late September, height is as closely linked with yield as percentage leaf nitrogen, and years with high initial nitrogen tend as early as September to be characterized by taller plants than normal. This is clear from the partial regressions, for these factors together account for little more yield variance than either separately.

After thinning, the plants develop rapidly and flower-buds are evident from early October. During October the amount of vegetative growth, whether measured by height of main stem or by amount of dry matter, is correlated with final yield, the larger the plant the higher the yield. An unexpected association is found in amount of defoliation and yield, slight leaf-shedding being associated with high yield, and premature shedding of the lower leaves with yield much below normal. Defoliation is significantly more highly correlated with yield than height itself, and there is no increase in correlation when both height and defoliation simultaneously are considered. Years of tall plants are thus characterized by restricted or delayed leaf shedding, while in years when the plants are stunted defoliation starts early and is heavy. The importance of defoliation and the part played by blackarm disease will be

referred to again later. Meanwhile it is interesting to note that as defoliation is here measured by numbers of leaves shed from the main stem, the rate of loss of leaves is more important for yield than the rate of their production, for correlation with the numbers of leaves produced on the main stem (node numbers), was at no stage significant.

Even when flowering is in full progress, during November, cumulative flower number is no more closely related to yield than is amount of vegetative growth. Again there is no closer agreement with yield by considering variates simultaneously; seasonal differences in vegetative growth are evidently followed later by closely similar differences in flower production. The rate of flowering is thus determined by plant size. Although by the end of November more than half the total flowers have opened, defoliation at this time is significantly more closely related to yield than is flower number itself—defoliation differences accounting for 87 per cent. of yield variance as compared with 64 per cent. for flower number. When the two variates are considered together the percentage is increased only to 90 per cent. Years of few flowers show premature leaf shedding while those of abundant flowering are characterized by long sustained activity of the lower leaves.

Unfortunately defoliation was not measured regularly in December, so that this variate must be excluded from regressions based on plant development at the start of harvest. Comparing stem dry weight and flower numbers, the simple regressions of each separately accounts for 88 to 89 per cent. of the yield variance, the correlation of yield with flower number increasing relative to that with dry weights after the peak of flowering had passed. Thus in years when dry weights are above normal flowering is more extensive and is continued longer. Height in these later months proves inferior to dry weights and flower numbers as an index of yield, at no time accounting for more than 76 per cent. of yield variance. This arises from differences in lateral branch development from season to season, plants of similar height being more bushy in habit in some years than in others.

DISCUSSION

(a) Predetermination of yield.

Summarizing these results it is found that when a fixed sowing date and the same cotton variety are employed plant size is the predominating factor determining yield production, whether size is measured by height, amount of dry matter, or flower number. The yield variation from year to year can from the earliest stage be related to seasonal differences in development.

It may appear at first sight that large plants must necessarily produce large yields, but with cotton this is frequently not the case. In Egypt (Crowther, 1937) in experiments distributed over the Nile Delta and Middle Egypt and covering several years, no correlation was established between final plant height and yield. The cultivator there dislikes an unusually leafy crop because

it may lack bolls. These conditions arise through the liability of cotton to shed flower buds when the environment is unfavourable, the products of assimilation being then diverted to increase vegetative growth. In the Gezira such disturbances seldom occur and are unimportant at the Gezira Research Farm.

Balls (1917) was the first to draw attention to the extent to which fluctuations in growth are 'not necessarily the result of circumstances which were contemporary with the manifestation, but may often be due to circumstances which acted weeks or even months before'. The present experiment gives ample evidence of this. The significant correlations with yield from germination onwards show that predetermination of yield may start almost as soon as the seed is sown and continue without interruption thereafter, final yield resulting from the integration of successive influences. In the case of the Gezira it seems in general true that the later in development the influence occurs the less is its importance for yield. Here, however, caution must be observed, for the early correlation of leaf nitrogen with yield does not imply that conditions within the plant immediately after germination are critical for yield; rather, it may be regarded as a sensitive index of a soil condition whose influence may be exerted continuously from germination onwards through the main growth period.

(b) Nitrogen supply.

Since seasonal differences in leaf nitrogen during August and September correspond with differences in amount of vegetative growth in October and November and in turn with final yields, rate of absorption of nitrogen by the crop clearly plays an important role in yield determination. This rate of absorption depends upon a combination of at least two factors, the concentration of available nitrogen in the soil and the total extent of the root-absorbing surface. Thus improved physical conditions of the soil as well as higher nitrogen supply may lead to more rapid nitrogen absorption. It is impossible with existing information to reach any more definite conclusion as to the nature of the 'soil factor'. As will be shown later (see Fig. 5) the yields in this experiment have increased rapidly and progressively in recent years without a corresponding increase in contiguous cotton areas, a difference ascribable to the residual effects of the previous leguminous crop and of ammonium sulphate. This is supported by the soil nitrate data of Green (1939) and emphasizes the importance for yield of available nitrogen. On the other hand, differences in internode lengths, which are correlated with those of yield, imply critical variation in water-supply from season to season, for it has been shown for the Gezira that extension growth, which determines internode length, is primarily a function of water-supply while node numbers depend upon the amount of nitrogen (Crowther, 1934). Heath (1937) found this applied also to his data for rain-grown cotton in South Africa. As irrigations on the observation plot were made at regular intervals in all years and were of

similar amounts, seasonal differences in water-supply must arise from changes in soil permeability. Thus the 'soil factor' at the present stage of the investigation must be considered as a combination of both amount of nitrogen and degree of soil permeability.

(c) Defoliation.

The value of defoliation as an index of yield has been abundantly demonstrated, but why this should be so is not at once clear. It is not primarily associated with the resultant change in leaf weight, for the correlation of defoliation with yield is consistently of a higher order than that of total amount of leaf dry matter present. Also experiments in which leaves were deliberately excised, and the loss of synthesized products thus made greater than in the normal senescence of leaves, showed no appreciable change in final yield, extra leaves higher up the plant replacing those excised (Crowther, 1934). The importance of defoliation apparently lies in its value as indicator of the influence in any season of two distinct factors each linked with amount of final crop. It is indicative of changes within the plant following the onset of fruiting, which may lead to premature leaf senescence; secondly it records the extent of any serious damage to the leaves by external agencies, for example by blackarm.

Dealing first with premature senescence, this arises from internal starvation (Gregory, 1928), consequent on the demands on the products of assimilation in the leaves by the developing fruits. The time of onset of senescence is intimately connected with plant size, for small plants produce their crop at comparatively low nodes on the main stem and so mature early. With large plants, producing bolls higher up the stem on later-formed nodes, the crop ripens later. Thus a correlation with defoliation automatically takes into account differences in plant size.

As an index of leaf damage defoliation at the Gezira Research Farm usually measures the extent of blackarm attack. This disease (Massey, 1931) starts as 'angular leaf spot' and usually develops into watery lesions on the petioles, stem, and, when severe, on the fruits. Premature leaf shedding follows and flower buds and bolls may also be destroyed.

The incidence of blackarm each year may be judged from Fig. 4 (a), giving the proportion of holes showing infection on field examination. It will be seen that there is great seasonal variation. This blackarm incidence is closely associated with amount of leaf-fall year by year, for, taking the mean percentage of holes infected from mid-September to end-October as a measure of blackarm severity, the correlation with November defoliation is $+0.782$, which exceeds the $P < .01$ significance level.

(d) Seasonal variation in the yield of the experiment.

Attention has already been drawn to the high yields of the experiment in recent years and the indication that they have arisen through progressive
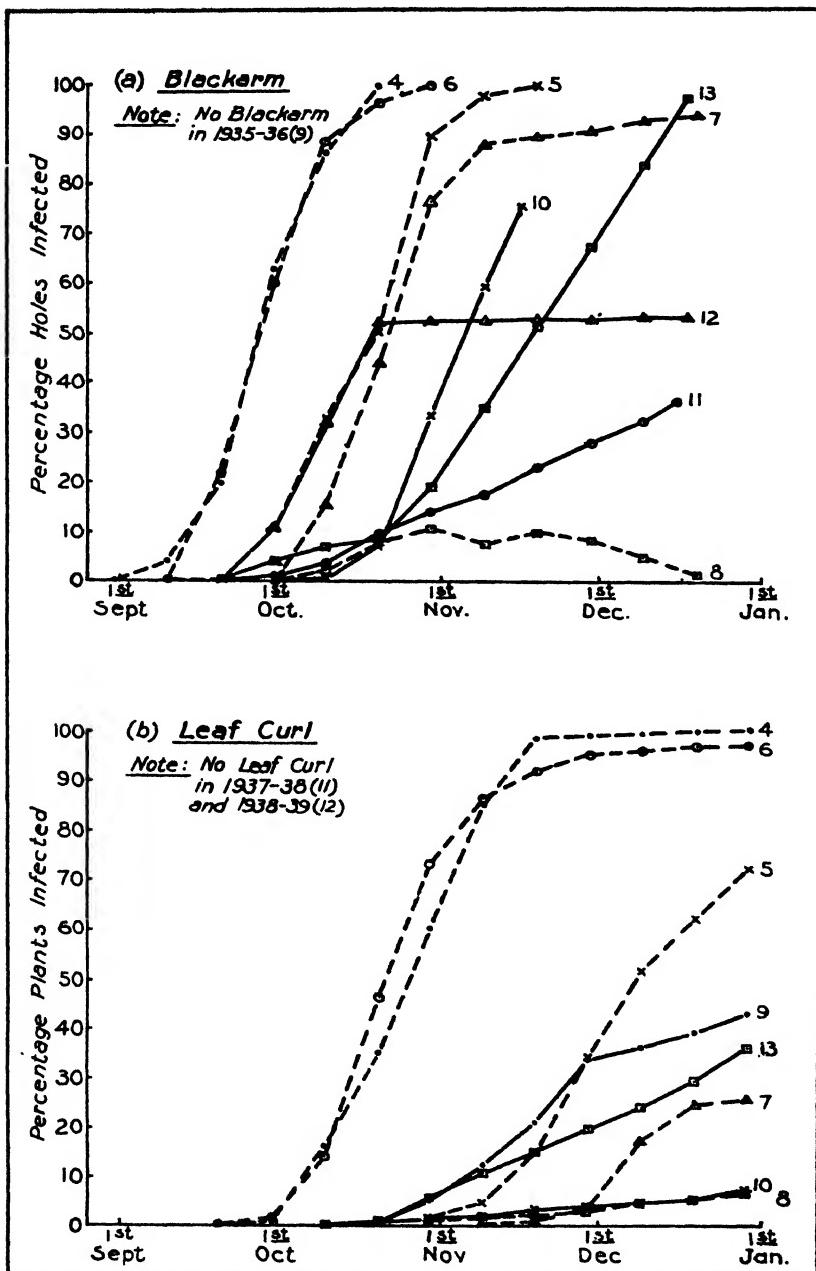
FIG. 4. Graphs showing (*a*) amount of blackarm infection and (*b*) amount of leaf-curl infection in separate seasons. For key see Fig. 1.

increase in soil nitrogen from manurial residues. With regard to the low yields the early leaf nitrogen correlation with yield implies that here, also, a soil factor must be held primarily responsible. Yet the importance of defoliation and the extent of blackarm incidence may appear to throw doubt upon this predominating influence of the 'soil factor'. Blackarm damage was the most obvious cause of yield failure in these unfavourable seasons, when the crop was examined at a late stage in the field. Considering the ten seasons together, the correlation between blackarm incidence and yield is -0.651, which is significant at the $P<.05$ level. Thus this evidence considered alone might be regarded as conclusive. But the early growth data are at variance with this interpretation. *The association between potential vegetative vigour, as expressed by percentage of leaf nitrogen at the end of August, and final yield is established with statistical significance before blackarm infection is observed on any plant in any year.* These field observations could not fail to detect blackarm incidence severe enough to cause interference with growth. Even a month later when both leaf nitrogen and crop height were significantly correlated with yield, blackarm, by this time extensive in two of the ten seasons, was still mostly at the angular leaf spot stage and so not likely to have interfered seriously with main-stem or root growth. Thus although plants in years of low yield usually have severe blackarm, its damage cannot be held primarily responsible for the crop failure. A possible explanation is that with the 'soil factor' unfavourable vegetative growth is not vigorous and the plants have increased susceptibility to disease. But it seems more likely, in view of the spread of blackarm by rain and of the adverse effect of rainfall on yield (Crowther and Crowther, 1935), that both lack of crop vigour and severity of blackarm arise independently from a common cause.

Leaf curl, which is widespread in some years, can also be dismissed as of an importance secondary to the soil factor and by the same argument. The percentage of plants showing symptoms of this virus disease are given in Fig. 4 (*b*) and, as with the blackarm data, in the two years with the lowest final yields the incidence was earlier and finally more widespread than in years of higher yield. Yet leaf curl never appeared before late September, by which time the inferior vegetative growth in the years of low yield was already apparent. The cause of the marked fluctuation in leaf-curl virulence from year to year is not known, but as it is spread by an insect vector (Kirkpatrick, 1931) rainfall may here also be responsible. Heavy rainfall, increasing the growth of both cotton ratoons and of weeds, allows of more foci of infection when the insects migrate from weeds to cotton.

(e) Seasonal fluctuation in Gezira yields.

To be of agricultural value the yields of an observation plot must fluctuate from season to season in a manner similar to that of the large-scale cultivation which it is designed to represent, for only then is it possible to apply to the second the conclusions drawn from the first. The yields of the experiment
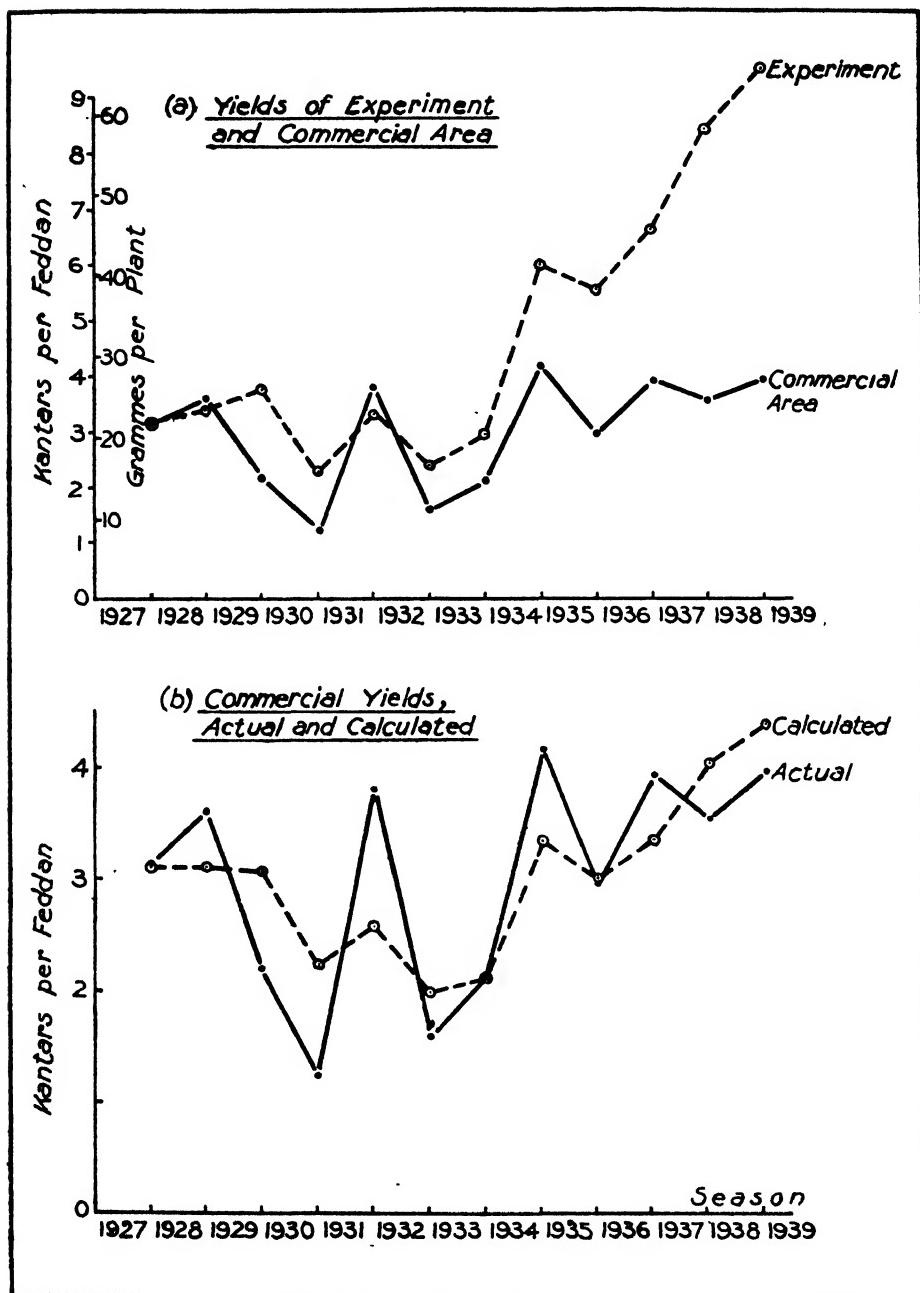
FIG. 5. Graphs showing comparisons of (a) yields of experiment and commercial area, (b) actual yields of commercial area with yields calculated from multiple regression equation of yield of experiment and time.

and of an area of 22,000 feddans (Taiyiba, Galil, Nidiana, and Wad Sulfab blocks) of commercial crop grown by Sudan Plantations Syndicate in the adjoining districts are plotted in Fig. 5 (a).[1] This commercial total comprises all the cotton of Sakel variety grown within 30 miles of the experiment, throughout the run of ten years.

Both experimental and commercial areas produced minimum yields in seasons 1930–1 and 1932–3. In recent years the observation plot has had a sequence of progressively increasing yields without any parallel increase on the commercial area, and the curves have consequently diverged markedly. This sequence of higher yields, as stated earlier, has arisen from improved soil fertility consequent upon accumulating residual effects of leguminous cropping and of ammonium sulphate dressings. The commercial area is cropped on a different rotation and manuring is practised only to a limited extent.

To allow for the progressive improvement of the experimental as compared with the commercial area, yields for the latter have been calculated from the former, by a regression equation which includes a linear term for change in yield with time. The equation is

$$y = +0.0675x_1 - 0.152x_2 + 1.78,$$

where y represents the commercial yield, x_1 the experimental yield and x_2 the serial number of the season: it is shown graphically in Fig. 5 (b). The coefficient of x_2, for change in yield with time, indicates that over the twelve seasons the yield of the experimental area increased by 0.152 kantars per feddan per annum, or a total improvement of 1.8 kantars which is equivalent to 60 per cent. of the mean commercial yield. The superiority of the 'standard practice' is agriculturally of considerable importance and is being followed up.

Comparing the two graphs of commercial yields in Fig. 5 (b), one actual and the other calculated from the regression equation, it will be observed that in general there is fairly close agreement. In the first two years both experimental and commercial areas gave medium yields. In the next year, 1929–30, there was divergence but each area had a low yield in 1930–1, 1932–3, and 1933–4, with higher yields in 1931–2. After 1933–4 there has been a succession of medium to high yields in both areas. Thus it may be concluded that over the period as a whole, the yield of the experimental area, subject to modification for progressive change in fertility, follows fairly closely that of the commercial cultivation. The explanations already advanced for the major yield fluctuations of the experiment may therefore be extended to the central Gezira area as a whole.

One further point of interest arises from the comparison in Fig. 5 (b). The fluctuations in the two curves in most cases correspond in direction season by

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[1] The two vertical scales in Fig. 5 (a) could be made to correspond, for although the yield of the experiment is expressed as gm. per plant, yields were obtained from the whole experimental area and were recorded in kantars per feddan.

season but differ in magnitude, being greater on the commercial area. At first sight this is surprising, for it would be anticipated that the smaller area would be the more variable, local influences averaging out on a larger scale. That this is not so is testimony to the maintenance of the 'standard practice' and to the greater control feasible under experimental conditions. Heavy rains may lead to flooding, delay in sowing, and excessive weed growth. On a small area flooding can be overcome by drainage and weeds removed while they are still small. Yet 'standard practice' is not necessarily the best agriculturally in any individual season. For example, the high yield of the commercial crop in 1931-2 is without an equivalent rise in the experiment. Here further analysis of commercial yield and practice should prove fruitful.

(f) *Forecasting yield.*

Since the experimental and commercial areas show correlated fluctuations in yield from year to year, and the yields from the experiment are in turn correlated with seasonal differences in early vegetative growth, material should be available for forecasting the Gezira yield at intervals throughout the growing season. But such crop forecasts will only be of value agriculturally when they predict the yield within prescribed limits and with a stated degree of reliability. Otherwise they are no improvement upon the individual estimates of the commercial cultivation based on visual inspection. Ten years must rank as too short a period on which to base a forecast of the Gezira as a whole expressed in kantars per feddan. At best, an estimate can be made of the yield of the experiment on a statistical basis and this applied in general terms to the surrounding commercial area to indicate whether the yield is likely to be far removed from normal and in what direction.

For the observation plot, forecasts may be made from the end of August onwards and as this covers the whole growing season it is impossible to use a single formula or the same combination of variates throughout. In early growth, percentage leaf nitrogen stands alone. Later crop height, defoliation flower number, and dry matter are all available. Because of the high degree of intercorrelation among these variates nothing more elaborate than a simple equation based on one of these variates only, for example defoliation, may be justified by their statistical significance. At the same time in any one season an estimate derived from several related variates would appear less liable to large error. Equations for a single variate in August and for two variates in subsequent months are given in Table VI, the units being the same as used earlier in the paper.

The accuracy of these forecasts of yield may be gauged from the magnitude of the residual standard error, also included in Table VI. The mean yield of the experiment over ten years was 36·2 gm. per plant, with a standard deviation for one season of 17·7 gm. Thus with a normal distribution the yield is likely in two seasons out of three to be within 17·7 gm., or about 50 per cent., of the mean. This statement is based solely upon the final yields of the ten
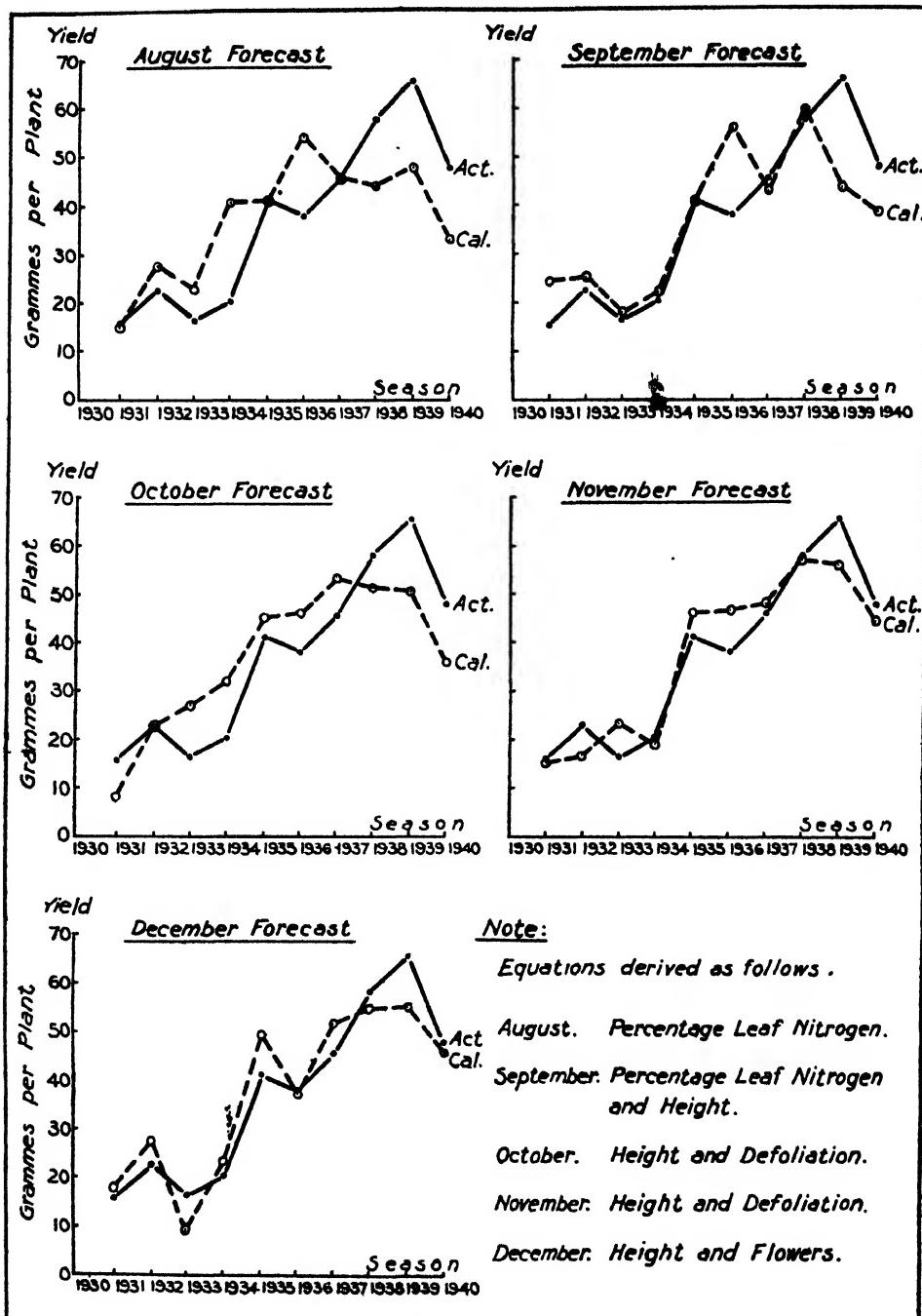
FIG. 6. Graphs showing yields of the experiment both actual and those forecast from monthly regression equations.

TABLE VI

Equations for Forecast of Yield

Month.	Variate.	Equation.	Residual standard error.
August	Leaf nitrogen (%)	$y = +24 \cdot 65n - 79 \cdot 9$	13·57
September	Leaf nitrogen (%) and height	$y = + 1 \cdot 679n + 31 \cdot 12h - 178 \cdot 4$	11·80
October	Height and defoliation	$y = + 0 \cdot 0438h - 11 \cdot 58d + 90 \cdot 9$	11·08
November	Height and defoliation	$y = + 0 \cdot 295h - 3 \cdot 763d + 53 \cdot 5$	6·57
December	Height and flower number	$y = - 0 \cdot 108h + 2 \cdot 052f - 4 \cdot 2$	6·71

y = yield estimate; n = percentage leaf nitrogen; h = height; d = defoliation; and f = flower number.

seasons. When forecasting from the data the standard error of the yield-esti-mate decreases according to the degree of correlation between the particular growth record and the yield. For example, in August the standard error is 13·6 gm. whereas by December it has been reduced to 6·7 gm. per plant. In two years out of three the yield of the experiment is likely to be within 6·7 gm. of that calculated from the December equation. Or in the case of a season where a yield near the average is predicted, the limit is about 18 per cent. above or below the forecast.

The actual yields of the individual seasons and those based on the monthly forecasts are shown in Fig. 6. All the monthly forecasts failed to indicate the exceptionally high yields of 1937–8 and 1938–9, and examination of Figs. 1 to 3 shows that these two seasons were characterized by growth and fruiting prolonged later than usual. The earliest forecast, in August, has not been reliable in recent years. This is due to percentage leaf nitrogen in young plants rarely exceeding 5·5 per cent. of dry matter even under the most favourable conditions. As an index, therefore, it may indicate years of low yields, but cannot differentiate between those likely to produce medium and those of high yields.

SUMMARY

An observation plot was started at the Gezira Research Farm thirteen years ago by A. R. Lambert, to study seasonal fluctuations in growth and yield of cotton in the Sudan Gezira Irrigation Scheme. The development of the plants and the yields are described and they are reviewed first in relation to predetermination of yield by seasonal influences during growth, and secondly to the underlying causes of Gezira yield fluctuations.

Significant correlation was found between the leaf nitrogen (as % of dry weight) within two weeks of sowing and the final yield four to seven months

later. In years in which leaf nitrogen was high shortly after germination the crop grew vigorously and was both taller and produced a total dry weight greater than normal. Flower numbers were also high in these years of vigorous growth.

Defoliation, measured by the number of leaves shed from the main stem, proved to be more highly correlated with yield than all the other growth characteristics observed. Heavy leaf shedding three to four months after sowing was regularly followed by low yields: where the lower leaves persisted longer than usual yields were correspondingly higher. Apparently defoliation is an index not only of crop size, a vigorous crop maturing later than one of stunted growth, but also of any subsequent damage to the leaves. In the experiment blackarm (*B. malvacearum*) was frequently severe and was responsible for part of the defoliation.

The experiment provides critical information on the causes of seasonal yield fluctuation. Although both blackarm and leaf curl were severe in years of low yield, the data collected early in the season proved growth to be inferior *before* the disease had become widespread. It is concluded that a 'soil factor', with which the amount of nitrogen available for the crop is closely associated, is primarily responsible for the major yield fluctuations and that the role of both blackarm and leaf curl is secondary.

The yields of the experiment from season to season correspond fairly closely to those of the surrounding commercial area, if allowance is made for a progressive improvement in the soil fertility of the experimental area resulting from crop and fertilizer residues.

Equations are given for forecasting the yield of the experiment at monthly intervals from shortly after sowing onwards.

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The Cytology of some Wild Species of Hordeum

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With fourteen Figures in the Text

INTRODUCTION

THE cytology of wild barley grasses has been described by many previous authors, but cytologists have sometimes failed to agree as to the number of somatic chromosomes in a species. This might be due to different species having been given the same name, or to interspecific changes affecting the number of chromosomes that survived in different ecological conditions.

Ghimpu (1932) has given a summary of chromosome counts of various Hordeum species by several authors. The somatic chromosome number has been stated to be 14 and 28 in *H. murinum*; 14 and 42 in *H. nodosum*; 14 in *H. pussillum*; 14 and 28 in *H. jubatum*; 14 in *H. gussoneanum*; and 28 in *H. bulbosum*. Kuckuck (1934) and Berg (1936) have also found 28 in *H. bulbosum*. In the meiotic metaphase *H. jubatum* L. was stated to have 14 and *H. nodosum* L. 21 bivalent chromosomes (Griffee, 1927); in *H. bulbosum* L. ring and chain quadrivalents were found (Berg, 1936).

The authors cited by Ghimpu (1932), with the exception of Griffee (1927), did not state their bases of nomenclature, i.e. whether the species were Linnean or of some other system. As will be shown later the classification in the past has been very confusing, so that the species with the same name studied by different investigators were not necessarily the same.

CLASSIFICATION AND CHROMOSOME NUMBERS

The classification of barley has been quite confusing, partly because of the different schools in taxonomy and partly because of physiological changes in the species. Thus *H. nodosum* L. was described as *H. pratense* Huds. (Britton and Brown, 1896), as *H. pratense* Huds. and *H. secalinum* Schreb. (Horwood and Gainsborough, 1933); *H. depressum* (Scribn. and Smith) Rydlb. was

described as *H. nodosum depressum* Scribn. and Smith (Hitchcock, 1935); *H. secalinum* Schreb. was described as *H. nodosum* L. (Rouy, 1913).

To avoid confusion, some morphological differences of the species investigated by the writer are given below.

1. *H. nodosum* L. ears of this species were received from the U.S. Department of Agriculture. There were two forms and one proved to be diploid (No. 1) and the other a tetraploid (No. 2). The differences between the two forms are shown in the following table:

	No. 1, Diploid.	No. 2, Tetraploid.
Lemma of central spikelet . . .	10 mm. long, 1 mm. wide	6 mm. long, 1·5 mm. wide
Reduced glumes of central spikelet . .	1·5 cm. long	1 cm. long
Reduced glumes of laterals (awn-like) .	1·5 cm. long	1 cm. long

Otherwise, no disagreements were found with the descriptions of previous taxonomists (Hitchcock, 1935; Britton and Brown, 1896; Pammel, 1901–4; Abrams, 1923; Jepson, 1925). The species was previously reported to have 14 and 42 chromosomes, and bivalents numbering 21 were found by Griffee.

The writer found 7 bivalents in the diploid and 14 in the tetraploid; the first has one pair of chromosomes with satellites and the second two pairs with satellites (Figs. 1 and 2). It is interesting to note that the tetraploid form was obtained at an elevation of 4,000 ft. in California, while the diploid was obtained from sea-level from the same State.

2. *H. jubatum* L. showed different lengths of awns for different times of sowing. Those sown in the winter and kept in the greenhouse until the next spring showed much longer awns and reduced glumes. This species had been described as perennial (Hitchcock, 1935; Jepson, 1925) and annual or biennial (Pammel, 1904). The present material is annual, but the morphology agrees with previous descriptions (Hitchcock, Jepson, Britton and Brown, Pammel, Abrams). The species has been stated to have 14 and 28 chromosomes.

The writer found 28 somatic and 14 bivalent chromosomes in the mitotic and meiotic metaphase respectively.

3. *H. bulbosum* L. The species, being distinguished by its bulb formation, the descriptions of previous authors showed no discrepancies (Coste, 1906; Husnot, 1896–99; Kuckuck 1934). It has 28 somatic chromosomes with 2 pairs possessing satellites (Fig. 3). The plates of both Ghimpu (1932) and Kuckuck (1934) showed merely 28 bars without even centromeres; this might have been due to fixation.

4. *H. murinum* L. One form of this species (No. 1) grows wild in Cambridge, England, and another (No. 2) was received from the U.S. Department of Agriculture. The outer glumes of No. 1 are reduced to two pubescent bracts with awns. The median lemma possesses an awn about 3 cm. in length. The lateral spikelets also possess an awn, and each spikelet possesses in addition two awn-like appendages growing out from the base. No. 2 differs from No. 1 in the following characteristics.

i. The colour of the ear is entirely brown.

ii. The lemmas of the lateral spikelets are about twice as long as those of the median.

iii. The size of the flower is greater than in No. 1.

iv. The awns of the median and the lateral spikelets are about the same length, while in No. 1 the median awn is twice as long as that of the lateral spikelet.

Except for the above points, the morphology agrees generally with the previous descriptions (Hitchcock, Jepson, Britton and Brown, Abrams). The species has been stated to have 14 and 28 somatic chromosomes.

The writer found 28 somatic chromosomes for both form No. 1 and form No. 2 (Figs. 4, 5). Both forms show two pairs of chromosomes with satellites. In the first meiotic metaphase both types display 14 bivalents (Fig. 7).

5. *H. gussoneanum* Parl. is commonly named Mediterranean barley. The writer found its morphology agreed with the descriptions of Hitchcock, Jepson, and Abrams. It was reported to have 14 somatic chromosomes.

The writer found 28 somatic chromosomes and two pairs with satellites (Fig. 6). The meiotic metaphase shows 14 bivalents.

6. *H. pussillum* Nutt. This species is called 'little barley'. It was described as a diploid and the present study confirms this. In the mitotic and meiotic metaphases 14 somatic chromosomes and 7 bivalents respectively are found.

The cytological findings are summarized in Table I.

TABLE I

Species.	Somatic chromosome number ($x = 7$).	
	Present investigation.	Previous authors.
H. bulbosum . . .	4x	4x
,, gussoneanum . .	4x	2x
,, nodosum . . .	2x, 4x	2x, 6x
,, murinum . . .	4x	2x, 4x
,, jubatum . . .	4x	2x, 4x
,, pussillum . . .	2x	2x

With the exception of *H. bulbosum* and *H. pussillum* every species shows more than one form, especially *H. nodosum*. The diploid and the tetraploid of the latter differ very little and they are very different from the other two-rowed species, *H. jubatum* and *H. gussoneanum*. Even if we ascribed the possible polyploidy in the other species to different methods of classification we can get evidence in *H. nodosum* that there is a polyploid series within the species.

One fact that attracted the writer's attention is that all the cultivated diploid species of Hordeum possess two pairs of chromosomes with satellites (Lewitsky, 1931). The writer spent much time investigating the morphology of the chromosomes of cultivated species. The forms studied, namely, *H. vulgare pyramidatum* Harlan, *H. deficiens gymnospermum* Kcke., *H. vulgare typica* L.,

H. vulgare trifurcatum Schl., *H. zeocrithum* L., *H. intermedium* Kcke., *H. spontaneum* C. Koch, *H. deficiens* Harlan, *H. distichon nutans* L., and the beardless six-rowed form from Silesia all without exception possess two
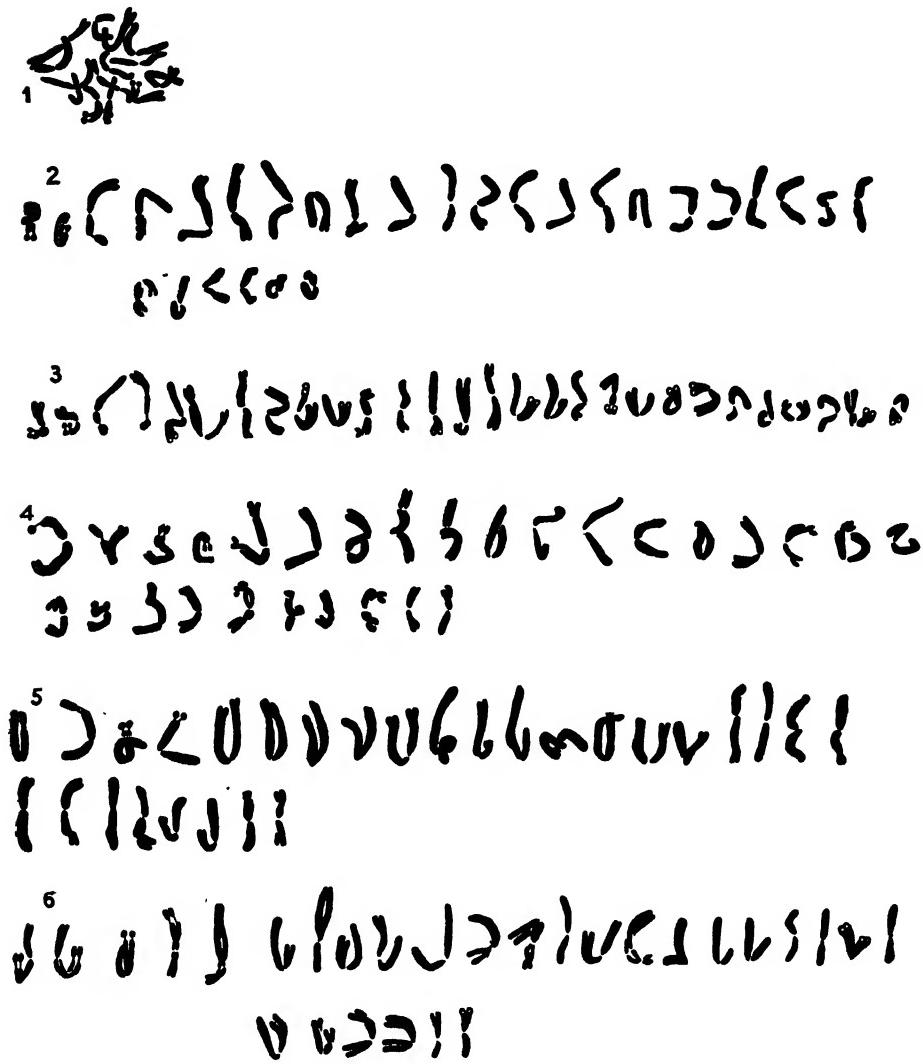
FIGS. 1–6. Somatic chromosomes, Fig. 1, *H. nodosum* L., No. 1; Fig. 2, *H. nodosum* L., No. 2; Fig. 3, *H. bulbosum* L.; Fig. 4, *H. murinum* L., No. 1; Fig. 5, *H. murinum* L., No. 2; Fig. 6, *H. gussoneanum* Parl. (× 900.)

pairs of chromosomes with satellites. The presence of one pair of satellited chromosomes in the wild diploid species and two pairs in the tetraploids, suggests that the phylogenetic relationship between the cultivated and wild species may not be close.

CELL SIZE

Investigations on the relation of the number of chromosomes and the size of pollen, and the distribution of stomata on the leaves are considered below.

TABLE II

Hordeum. Diameter (μ) of Pollen with S.E.

H. nodosum (2*x*)	38·70±0·097	*H. nodosum* (4*x*)	41·30±0·047
,, *spontaneum* (2*x*)	42·80±0·105	,, *jubatum* (4*x*)	39·00±0·053
		,, *murinum* 2 (4*x*)	46·60±0·086
		,, *murinum* 1 (4*x*)	52·80±0·076
		,, *bulbosum* (4*x*)	50·33±0·159
		,, *gussoneanum* (4*x*)	57·40±0·288

The size of pollen of the tetraploid *H. jubatum* has been previously given as 41·42 ±0·14 and of the hexaploid *nodosum* as 50·66±0·17 (Griffee, 1927) in comparison with a value of 43·4±0·21 in the cultivated diploid species *H. distichon*.

There seems, therefore, to be no relation between chromosome number and size of pollen. *H. murinum*, Nos. 1 and 2, though both are tetraploids, show greater divergence than *H. nodosum*, Nos. 1 and 2, a diploid and tetraploid respectively. Moreover, *H. jubatum*, a tetraploid, has smaller pollen grains than those of the diploid *spontaneum*.

TABLE III

Distribution of Stomata

Species.	Upper leaf surface.	Lower leaf surface.	Ratio upper/ lower.	Average number of stomata for upper and lower surfaces (in 0·094 sq. mm.)
H. spontaneum (2*x*) .	7·4±0·124	7·6±0·116	0·97	7·50
,, *nodosum* (2*x*) .	13·5±0·147	5·4±0·125	2·50	9·45
,, *nodosum* (4*x*) .	7·7±0·090	8·5±0·111	0·91	8·10
,, *jubatum* (4*x*) .	11·3±0·101	7·5±0·080	1·51	9·40
,, *bulbosum* (4*x*) .	9·9±0·078	8·6±0·048	1·15	9·25
,, *gussoneanum* (4*x*) .	9·7±0·079	6·7±0·067	1·45	8·20
,, *murinum* 2 (4*x*) .	7·4±0·104	4·7±0·195	1·57	6·05
,, *murinum* 1 (4*x*) .	5·4±0·119	5·4±0·141	1·00	5·40

The results show no relationship between chromosome number and the number of stomata.

In the higher plants' increased cell size has been proved by previous investigators to follow increase in chromosome number. Further, species probably change in size of cells as a result of gene changes. Thus species with the same number of chromosomes do not necessarily have the same size of cells.

MEIOSIS IN *H. BULBOSUM* L.

Berg (1936) found that *H. bulbosum* behaved as an autotetraploid giving a maximum association of seven quadrivalents. The writers' observations

confirm these conclusions; some configurations are shown in Fig. 8, and the results of twenty-one cells are shown in Tables IV and VII.

TABLE IV

Mean number of configurations per cell.				Mean chiasmata per cell.	
Quadrivalents.	Bivalents.	Trivalents.	Univalents.	Total.	Terminal.
4·2	5·52	0·05	0·05	26·14	25·62

Comparison between the results of the writer and those of Berg on the same species and the results obtained by Upcott (1935) on *Lycopersicum esculentum* are shown in Table V, and a comparison of the number of cells for different configurations are shown in Table VI.

From the results of Berg and of the writer it can be deduced that terminalization is the rule in this species and interstitial chiasmata are only exceptions. The failure of complete terminalization is probably due to structural changes in the chromosomes arresting the process of terminalization. As regards the configurations, the writer found three new types (new to bulbosum) namely the 'cross' quadrivalent with three chiasmata (Fig. 8a), the 'double triangle' quadrivalent with five chiasmata (Fig. 8b), and the 'double ring' quadrivalent with with six chiasmata (Fig. 8a). The 'double triangle' and the 'double ring' quadrivalents were not found in the tetraploid Lycopersicum, but Upcott found the quadrivalent types 2, 5, and 7 (of Table V). In the triploid Lycopersicum Upcott found trivalents of types 1, 2, and 3, but Berg and the writer found only the type 4 trivalent.

Table VI shows that the three kinds of quadrivalent configuration (namely types 1, 4, and 6) which are common to all three sets of results agree in frequency in the results of the writer and of Berg, but the results of Upcott are different. If the configurations of both the species were due to random association they should have agreed. As regards the bivalents, the three sets of results only agree in the types of configuration but not in their frequency. Upcott's results show that the rod and the loop bivalents occur nearly in a one to one ratio. The results of the writer and those of Berg were as follows:

	Ring bivalents with 2 Xta. per cent.	Rod bivalents with 1 Xa. per cent.
Berg	91·4	8·6
Chin	62·9	37·1

Only a small percentage of the bivalents are rod shaped in Berg's results, which therefore show a much higher chiasma frequency than the writer's, possibly due to environmental influences. This means that the difference between the Hordeum results and those of Upcott for Lycopersicum are not due to a difference in chiasma frequency but must be the result of some other factor, such as the much larger chromosomes of Hordeum.
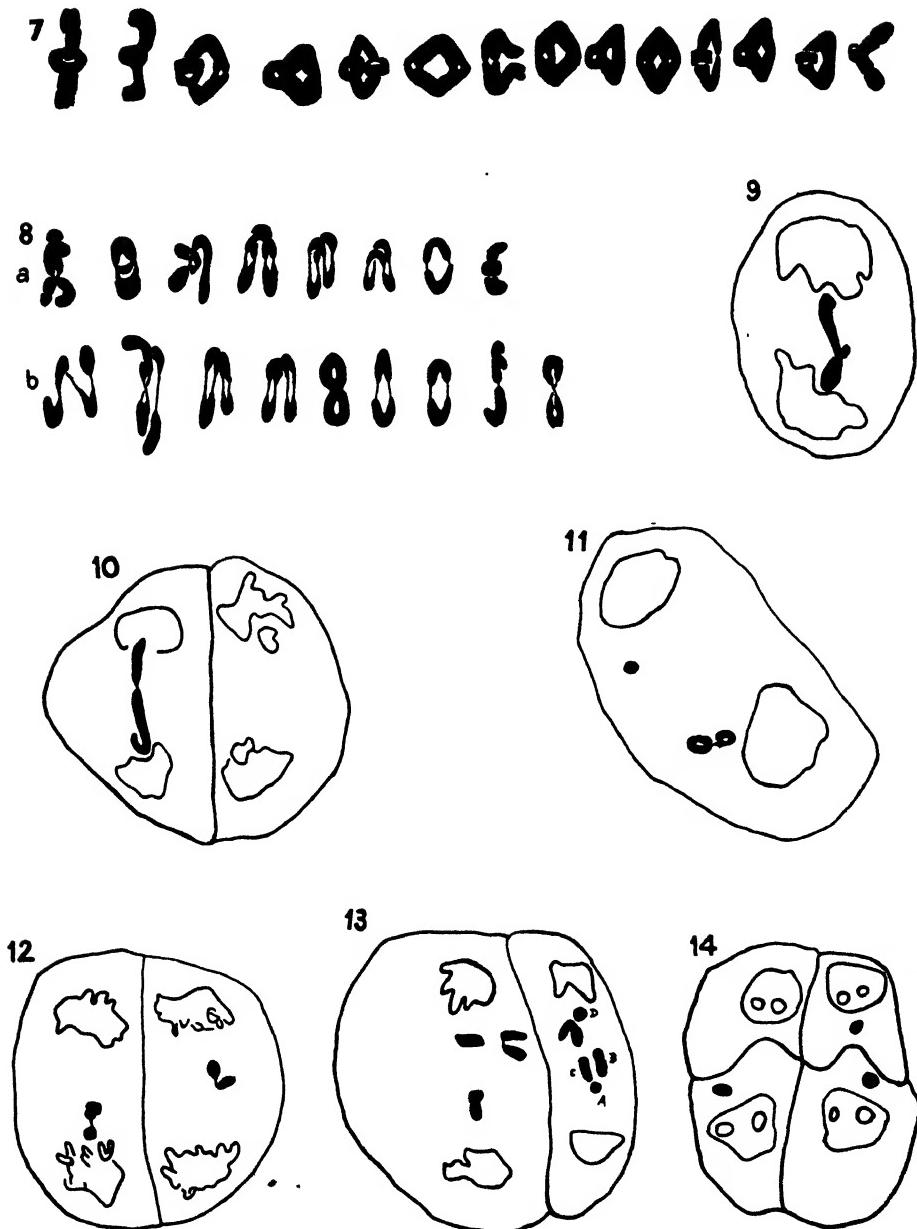
FIGS. 7–14. Fig. 7, Meiotic chromosomes of *H. murinum* L. (×1100.) Fig. 8, Meiotic chromosomes of *H. bulbosum* L. (×1100.) Fig. 9, first anaphase, and Fig. 10, second anaphase, in *H. bulbosum* L. showing bridges (×1100) and Figs. 11–14. Misdivision of centromere at meiosis in *H. bulbosum* L. (×1100.)

TABLE V

Comparison of Configurations

Configurations.	Bivalents.		Trivalents.				Quadrivalents.								
	type 1		type 1	type 2	type 3	type 4	type 1	type 2	type 3	type 4	type 5	type 6	type 7	type 8	type 9
Number of chiasmata	1	2	2	2	3	2	3	3	3	4	4	4	4	6	5
Lycopersicum:															
Upcott (1935) .	404	390	2	1	1	—	67	13	3	99	3	4	4	—	—
Percentage .	50·9	49·1	50	25	25	—	34·7	6·7	1·5	51·3	1·5	2·1	2·1	—	—
H. bulbosum L.:															
Berg (1936) .	66	700	—	—	—	10	96	—	—	620	—	18	—	—	—
Percentage .	8·6	91·4	—	—	—	—	13·1	—	—	84·5	—	2·5	—	—	—
Chin .	43	73	—	—	—	1	6	—	2	77	—	1	—	1	1
Percentage .	37·1	62·9	—	—	—	—	6·8	—	2·3	87·5	—	1·1	—	1·1	1·1

TABLE VI

Frequency of Types 1, 4, *and* 6 *Quadrivalents*

			$\bowtie$	$\Diamond$	$\circ--$
Upcott	.	.	67	99	4
Percentage	.	.	39·4	58·2	2·4
Berg	.	.	85	620	18
Percentage	.	.	11·8	85·8	2·5
Chin	.	.	6	77	1
Percentage	.	.	7·1	91·7	1·2

TABLE VII

Frequency of Quadrivalents in H. bulbosum *L.*

Configuration in cell.	Berg.		Chin.	
	Number of cells.	Percentage of cells.	Number of cells.	Percentage of cells.
7 IV	2	0·8	1	4·8
6 IV+2 II	49	19·8	4	19·0
5 IV+4 II	95	38·3	4	19·0
4 IV+6 II	76	30·7	6	28·6
3 IV+8 II	22	8·9	3	14·3
2 IV+10 II	1	0·4	2	9·5
1 IV+12 II	3	1·2	1	4·8
Total cells	248	—	21	—

Structural changes in the chromosomes as evidenced by the occurrence of bridges.

Four first anaphase bridges (Fig. 9) were found out of 93 cells, a percentage of 4·3; no fragments were found. The bridges are produced by crossing over in an inversion.

At second anaphase, 6 bridges were found (Fig. 10) among 90 pollen mother cells, a percentage of 6·67. No fragments are expected in the second division and none was found.

The species therefore shows a small degree of hybridity due to structural changes in the chromosomes. Unfortunately the pollen mother cells of different stages were not obtained from the same plant.

Abnormal splitting of univalents.

Nishiyama (1931) in a heterozygous fatuoid oat found that univalents lagging in the second division fragmented at the spindle fibre attachment, and Katayama (1935) found fragmentation of univalents in the second division of haploid monococcum. Upcott (1937) found four possible abnormal divisions of the centromere in Tulipa and, of these four, three took place in the first and one in the second division. Darlington (1939) in Fritillaria found abnormal division of centromeres in both divisions; he was the first to explain this phenomenon. Such misdivision of the centromere, to use Darlington's terminology, is due primarily to the instability of the centromere itself.

In the writer's material, *H. bulbosum* L., misdivision of the centromere was observed in both the first and second divisions. Fig. 11 exemplifies a three to one type in the first division; one separated from the remaining three and is moving to one pole, while the other three are near to the other pole. Fig. 12 illustrates misdivision of the centromeres in the second division; one univalent has probably divided normally in the first division to give two daughter chromosomes each of which has misdivided in the second.

Fig. 13 shows three half univalents in the left-hand cell, one of which is misdividing. The right-hand cell can be interpreted in two ways (*a*) as three half univalents of which one is undivided, a second has misdivided to give unequal arms B and D, and a third CA is in process of misdivision, or (*b*) it might also result from a four to none type at the first division, in which ABCD all went to the same pole, followed by a second division of the three to one type in which ABC go to one pole and D to the other.

Univalents lagging on the spindle may, after a misdivision either in the first or second division, form supernumerary nuclei (Fig. 14).

SUMMARY

Some taxonomic description is given of the wild species of Hordeum, the cytology of which is examined.

H. pusillum Nutt. and one of the two forms of *H. nodosum* L. each has 14 mitotic chromosomes and 7 bivalents in meiosis, one pair having satellites.

H. gussoneanum Parl., a second form of *H. nodosum* L., *H. jubatum* L., and two forms of *H. murinum* L. (from England and U.S.A. respectively) have 28 somatic chromosomes and 14 bivalents at meiosis, no multivalents being observed; each has two pairs of chromosomes with satellites. The diploid and tetraploid forms of *nodosum* came from different altitudes in California.

The numbers found do not always agree with those found by other authors, nor do these authors agree among themselves. This is probably due to polyploidy within the species, but may sometimes be due to differences in nomenclature since the taxonomy is very confused.

No relation was found between number of chromosomes and size of cells.

H. bulbosum L. with 28 somatic chromosomes behaves at meiosis like an autotetraploid, with a very small degree of hybridity as evidenced by the occurrence of bridges in both the first and second meiotic divisions. Misdivision of the centromere was observed in univalent chromosomes in both divisions. The chromosome configurations of the first meiotic metaphase have been compared with those obtained by other authors in autotetraploids.

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The Genetics of Embryo-Sac Development

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With Plates VI and VII and seven Figures in the Text

1. GENETICS AND EMBRYOLOGY

THREE, four, or five nuclear divisions stand between the mother cell and the egg in the development of flowering plants. The first two of these constitute meiosis; the remainder are simply mitotic. The whole series is determined in each plant as a developmental system on whose successful co-ordination the fertility of the plant and the survival of the species depends. So far as the system works with regularity and success we must therefore suppose that its determination is genotypic and its origin adaptive. But since total fertility is almost unknown amongst plants we must be prepared to find serious exceptions to regularity and success in the course of development. From what we know of pollen development these failures will arise from two causes: *first*, the regular segregation and recombination of differences which follows regular pairing and crossing-over at meiosis, and *secondly*, the irregular segregation which follows failure of regular pairing and crossing-over at meiosis.

Our main duty in all embryo-sac studies must therefore be to separate the maternally determined course of development from these immediate mechanical and genetical consequences of meiosis. The first may be characteristic of the species; the second will often vary from ovule to ovule.

Take first the mechanical effects of meiosis. The development of the embryo-sac has been studied in thousands of flowering plants, but in very few of these has any account been given of the chromosome constitution of the plant, of its actual or probable behaviour at meiosis, or of the corresponding processes on the male side, which would provide a warning of the occurrence of abnormality and a clue to its effects. Indeed plant embryology, apart from recent studies of apomixis, has passed its life in a state of isolation from the study of meiosis, and of its genetic consequences, which has been growing up alongside it. We know from pollen mother cell development that the number of nuclei and cells produced by meiosis may separately or together

vary within wide limits. We know that the viability of these products may vary in the same plant. We also know the circumstances of structural hybridity and polyploidy in which this variation has its most serious effects. If the embryologist does not take account of this irregularity he makes his already difficult task of the seriation of stages even more difficult. For example, the five cells in a row in Culcitium (Afzelius, 1924) and the extra nuclei and variable movement in *Tulipa praecox* (Bambacioni-Mezzetti, 1931) have puzzled their observers. The first is probably a structural hybrid, the second is certainly a triploid or numerical hybrid.

Secondly, consider the genetic effects of meiosis. The genetic control of development was established by Renner (1921) when he showed that in half the ovules of hybrid Oenothera species with two types of spore the micropylar spore was able to supplant the chalazal spore as the functional embryo-sac. There is thus a conflict between the maternal differentiation of morphology and the filial selection of genetics. This is in a sense a conflict between the daughter nucleus and the cytoplasm of the mother cell; in the extreme case the new genotype of the nucleus can override the effect of position or size of the cell in determining development.

Now variability as to which of the four cells shall function has been recorded in fifty-three families in many groups of Angiosperms as well as in Taxus and Gnetum amongst Gymnosperms (Schnarf, 1929; Chamberlain, 1935). But since we see that it depends on the occurrence of differences in genotype between the four spores, we must admit that it is a property not of one species as opposed to another so much as of a hybrid plant as opposed to a non-hybrid plant.

Doubtless the overriding effect of the genotype will be more marked in some groups than in others. Particularly, it seems to be more marked in the *pro-micropylar* development of Oenothera and Rosa than in the *pro-chalazal* normal development. On the other hand, where the differences of segregation are extreme, as in a monosomic plant ($2x-1$), and there is, as it were, no help from outside through a multicellular archesporium, we may predict that genotype will always override position. In a word, competition between spores will take effect wherever there are differences enough between them to make its action important.

In the absence of any knowledge of the chromosome constitution or genetic properties of his material, the embryologist can proceed indirectly. Beginning from the other end of development, he can rely on fertility. He can argue that where the embryo always fails the embryo-sac may well be abnormal. Coming events cast their shadow before them. Not merely the absolute but, after fertilization, the relative development of the two ends of the embryo-sac have been shown to be genetically controlled (e.g. Hiorth, 1926, on Oenothera). Yet many histories of development of the embryo-sac have been written in plants that never produce a seed. Sometimes the author is aware of this issue, though uncertain of its relevance (e.g. Anogra, Johannsen, 1931): fertile rela-

tives should be compared with the infertile plant. In other cases the author is unaware of the issue. The triploid *Tulipa praecox* is sterile, and being triploid would in any event suffer from the spatial derangements and even fusion of the meiotic products which Bambacioni describes as though they were peculiar to the maternal type of embryo-sac. Similarly another investigator has described the embryo-sac in *Ranunculus Ficaria* without saying whether he is dealing with a somewhat fertile diploid or the less fertile triploid, tetraploid, or hexaploid forms of this species (Metcalfe, 1939).

Another aspect of embryology of general importance is the ploidy of the endosperm. We assume from the evidence of development that a ploidy of two, three, four, five, or even fourteen is the normal condition in the endosperm arising from a particular type of embryo-sac. This assumption could be verified by direct observation of the chromosomes. One in a hundred accounts of endosperm development attempt to record this fundamental fact. The remaining ninety-nine are content with classifying the method of origin of the cell walls by a distinction whose value has never been revealed. Not only is the normal chromosome constitution of the endosperm important. The number must (as we shall see from abnormalities of meiosis) be more variable than morphologists have supposed, and the variations more significant than geneticists have realized. For example, Hiorth (l.c.), in a detailed account of the genetics of endosperm development in Oenothera, makes no mention of its being, as Renner showed in 1913, diploid and not triploid in this genus. Yet it is quite certain from Hiorth's observations that the precise balance of the endosperm (diploid or triploid) has been a condition of the balanced lethal system as we know it in the hybrid species of Oenothera.

The greatest profit is therefore to be gained in the future from linking embryo-sac studies with our knowledge of the genetical and mechanical consequences of meiosis. Our purpose in the present article is to indicate particular and disconnected examples of the way in which embryo-sac studies can be made use of in understanding these general relationships in certain Liliaceae.

2. MATERIAL

Embryo-sacs were studied in the following species (L, I, and C indicate whether the chiasmata were localized, intermediate, or complete at the first metaphase):

<div style="margin-left:2em">

Fritillaria: *F. ruthenica* (L)

F. latifolia 2x and 3x (L)

F. meleagroides (L)

F. Meleagris (L)

F. tenella (L)

F. Drenowskii (I)

F. involucrata (I)

F. pyrenaica (I)

F. pallidiflora (C)

</div>

                 F. libanotica (C)
                 F. imperialis (C)
                 F. pudica 2*x* and 3*x* (C).
     Lilium: *L. umbellatum* (C)
                 L. Thunbergii (I)
                 L. × *testaceum* (Frankel et al., 1940).
  Tulipa, Leiostemones: *T. lanata* (3*x*)
                 T. Gesneriana (3*x*×2*x* seedling, Inglescombe Yellow×
                   Bouton d'Or, 2*n* = 35, $10^{iii}+2^{ii}+1^{i}$ at M I).
     Eriostemones: *T. primulina* (no prefusion).

All of these forms have a tetrasporial embryo-sac and, apart from *Tulipa primulina*, a triple prefusion at the chalazal end. The diploid and triploid seedlings of *F. pudica* had sets of 12 with 2 M's, like that described by Sax (1918) and unlike our ordinary stocks of diploid and triploid with 13 as the basic number and one M type. This species must therefore contain two basic types.

The methods were those described by Darlington and La Cour (1940 and 1941), apart from Plate III, Fig. 11 (see legend).

3. Mother Cells in Prophase and Metaphase

In a *T. Gesneriana* seedling we have found twin mother cells without nucellar tissue between them (Pl. III, Fig. 10). Twin cells separated by a layer of nucellus have been found in *T. praecox* by Bambacioni-Mezzetti (1931). Their importance is in their ability to give fraternal twins (one of which may be parthenogenetic) or failing that to reduce the effects of sexual sterility.

In pollen mother cells of three species of Fritillaria the nucleolus comes to the surface of the nucleus during prophase. In another twenty-four species it retains its free and central position (Frankel, 1937). In yet another species, *F. pyrenaica*, we have found cap nuclei in both pollen and embryo-sac mother cells. But in five other species a new situation arises. Cap nucleoli are formed in the embryo-sac but not in the pollen mother cells. This is true regularly in *F. imperialis*, *F. libanotica*, and *F. Meleagris*, occasionally in *F. meleagroides* and *F. latifolia*.

The species of Fritillaria examined have various degrees of proximal localization of chiasmata in the pollen mother cell. Those marked in the list have the same degree of pairing (localized, intermediate, or complete). It is remarkable that a property which is so variable as between species should show such exact correspondence as between pollen and embryo-sac mother cells.

The spindle fills a smaller proportion of the cell but is, of course, much bigger in the embryo-sac mother cell than in the pollen mother cell, particularly in Fritillaria (Pl. I, Figs. 4 and 5). Its development also seems to be much slower. Thus double plates and plates with one or two bivalents far off them

are common. Farther the shape of the bivalents is greatly modified. Their pairs of centromeres are drawn farther apart. Indeed this distance seems to be proportionate to the length of the spindle (Text-fig. 1).
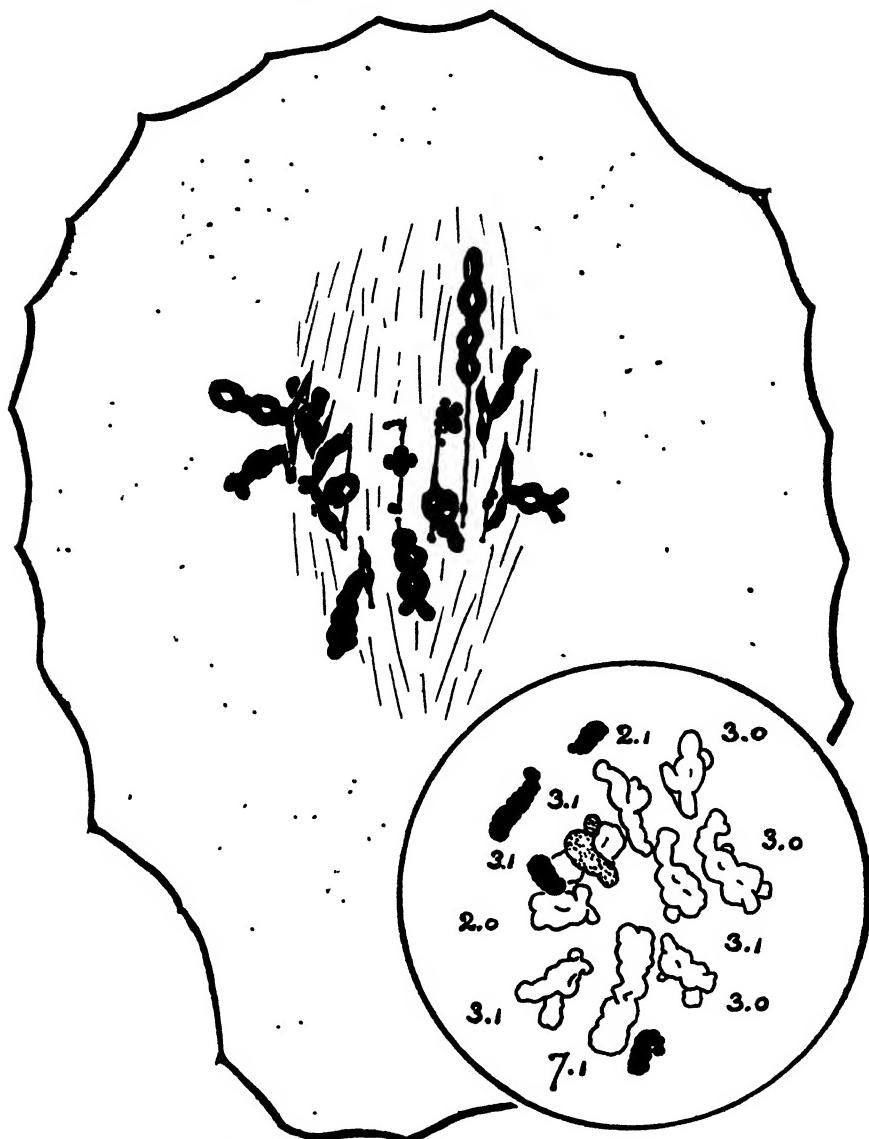
TEXT-FIG. 1. First metaphase in embryo-sac of *Fritillaria pallidiflora* with complete pairing. Inset: the same in *Lilium Thunbergii* var. Mahony with four univalents and interlocking. Total and terminal chiasma frequencies are given; 32/6 in all. All figures except 7 magnified 1,600 times.

The most important consequence of slowness in orientation at first metaphase is a frequent lack of synchronization at anaphase such as is not found in

pollen mother cells. Some bivalents have evidently not reached the plate at the time when others are already separating.

In *Lilium Thunbergii*, *L. umbellatum*, and the cross *L. testaceum* failure of pairing occurs, with a frequency comparable with that in the pollen mother
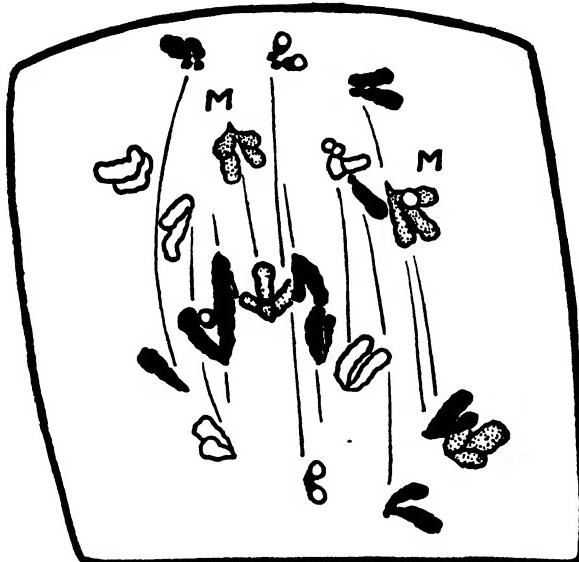
TEXT-FIG. 2. First anaphase in *L. testaceum*. One bridge and fragment. M, median-type chromosomes. Three daughter bivalents in next section.

cells and recorded elsewhere (Frankel et al., 1940). The univalents are disposed at random on the two sides of the plate and not concentrated on one side—with this modification that they often lie in pairs. The univalents sometimes undergo misdivision at the first anaphase, or their daughters at the second (Text-figs. 5 and 6). If at the first, three-armed chromosomes appear at the second.

In the first species, as so often happens, failure of metaphase pairing is correlated with a spatial derangement of the prophase as shown by the interlocking which results from it (Text-fig. 1). In the cross it is correlated as in the pollen mother cell with inversion crossing-over and the formation of dicentric bridges and acentric fragments (Text-fig. 2). The same evidence of inversion hybridity was also found, as is usual in triploids, in *Tulipa lanata* (Pl. II, Fig. 9). It may be recalled that such crossing-over occurs in more than 10 per cent. of pollen mother cells of *Tulipa praecox* (Upcott, 1937) and probably occurs likewise in embryo-sac mother cells.

The effect of bridges on the embryo-sac anaphase is different from that in the pollen mother cell. They are not more stretched by the stretching of the spindle, but less so. Instead of breaking they may twist the spindle and pull round the two separating groups of chromosomes so that they come to lie in

planes at right angles with the bridge joining the two nearest corners of the groups (Pl. II, Fig. 8). The effect of this contortion is seen at second metaphase (Text-fig. 4B). If, on the other hand, the contortion fails to occur the stress has a different effect which we shall see at the second division.

TABLE

Record of Lilium testaceum *after first Metaphase, M I*

E.S.M.C.				A I	M II	A II
Normal	.	.	.	7	2*	—
B+*f*	.	.	.	3	1	2
Fragments	.	.	.	—	2	4
Restitution	.	.	.	—	1†	1
Misdivision	.	.	.	—	1‡	1
Total	.	.	.	10	7	8

* One had potential Bᴵᴵ.　　† See text-fig. 4.　　‡ From A I.

4. SECOND METAPHASE

The second metaphase of meiosis is clearer in the embryo-sac than in pollen mother cells where large chromosomes are too crowded for thorough study. To begin with we may consider one pair of second division cells in detail; they show both mechanical and genetical consequences of abnormalities at the first division already mentioned. The plant is a cross of a triploid garden tulip (Inglescombe Yellow) by a diploid (Bouton d'Or). It has 35 chromosomes of which 17 have passed to one pole and 18 to the other. The drawing has been spaced (Text-fig. 3); the photographs have been taken at two focuses (Pl. II, Figs. 6 and 7).

There has been crossing-over between four pairs of relatively inverted segments, with the production of four acentric fragments (*f*). Two of the corresponding dicentrics have given first division bridges (Bᴵ). These bridges, instead of distorting the plates as a whole, control the second division orientation of the chromatids concerned in them. A third cross-over chromatid has formed a loop which will give a second division bridge (Bᴵᴵ). And the last has undergone non-disjunction in a trivalent, so that its bridge formation will depend on the chances of orientation of two separate centromeres (Bᴵᴵᴵ—the dicentric chromatid has been drawn broken to show it more clearly).

Another odd chromosome owes its shape to crossing-over between disparate though no doubt homologous segments. Two of its chromatids which should correspond are unequal in length. The conditions that would lead to this situation have already been described in Tulipa (Upcott, 1937).

Mechanically the embryo-sac metaphases confirm a suspicion we have long entertained about many pollen mother cell metaphases. The chromosomes are not in the stable and uniform condition that we find at all other metaphases of mitosis and meiosis (unless the short arms of one bivalent in Text-fig. 1 are an exception). In the tulip they are partly engaged in major spirals (L and R)

and partly stretched out in their simple and invisible minor coiling. This minor coiling is weaker than at mitosis for the chromosomes are longer and thinner. We find the same variability in coiling in diploid *Fritillaria pudica*. What is the cause of this variability? The frequencies, distributions, and
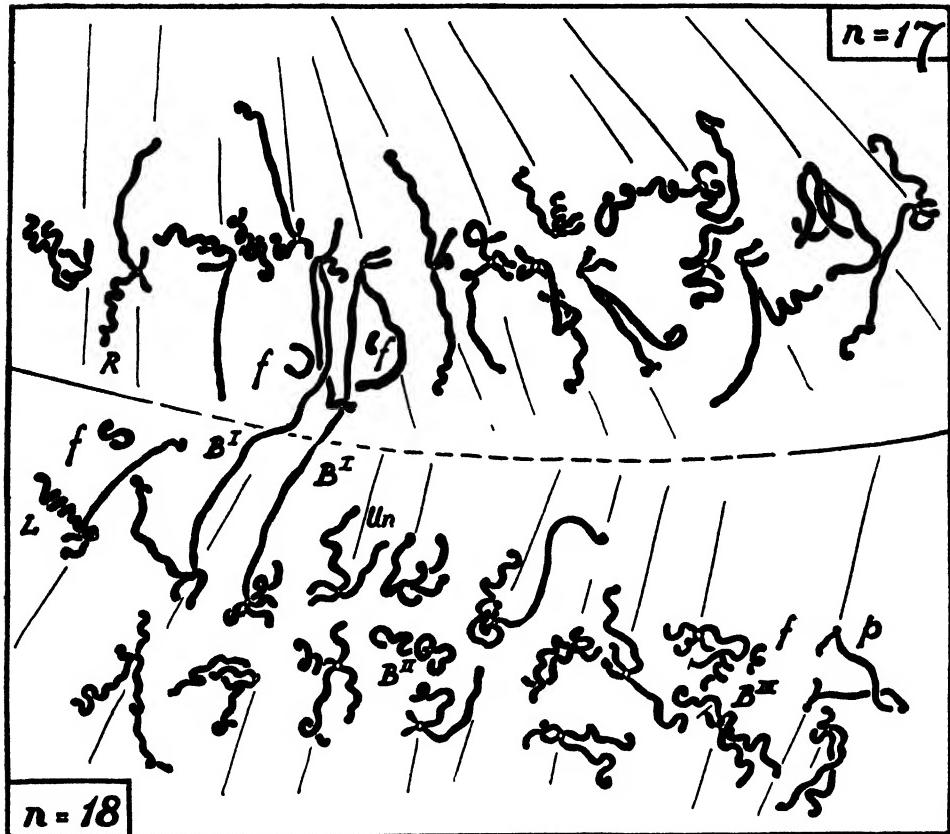
TEXT-FIG. 3. *Tulipa Gesneriana*, $3x \times 2x$, with 35 chromosomes (Inglescombe Yellow × Bouton d'Or). R and L, directions of coiling. B^I, B^{II}, B^{III} actual and potential bridges. *f*, acentric fragments. *Un*, unequal-armed chromosome. *p*, precocious chromosome (cf. Pl. I).

positions of the stretched parts suggest that they were stretched at the preceding anaphase. The asymmetry of coiling in chromatids would then be due to the asymmetry of chiasmata in trivalents. In other words we must suppose that the major spiral when pulled out cannot recover at the next metaphase, but if left alone will be capable of retaining its first metaphase coiling throughout the second division.

In the second metaphases of *L. testaceum* the spindle produces some marked distortion of the chromosomes of a kind not found in pollen mother cells. They are stretched wherever they lie axially in the central parts of the spindle. These parts must therefore be the most active in stretching at metaphase, just

as they are in anaphase. Indeed the whole situation suggests that the spindle is anticipating the movement of the chromosomes, If this is so, such distortions merely provide further examples of the chromosomes being sometimes too slow in forming the plate in a cell, and on a spindle, of unaccustomed size.
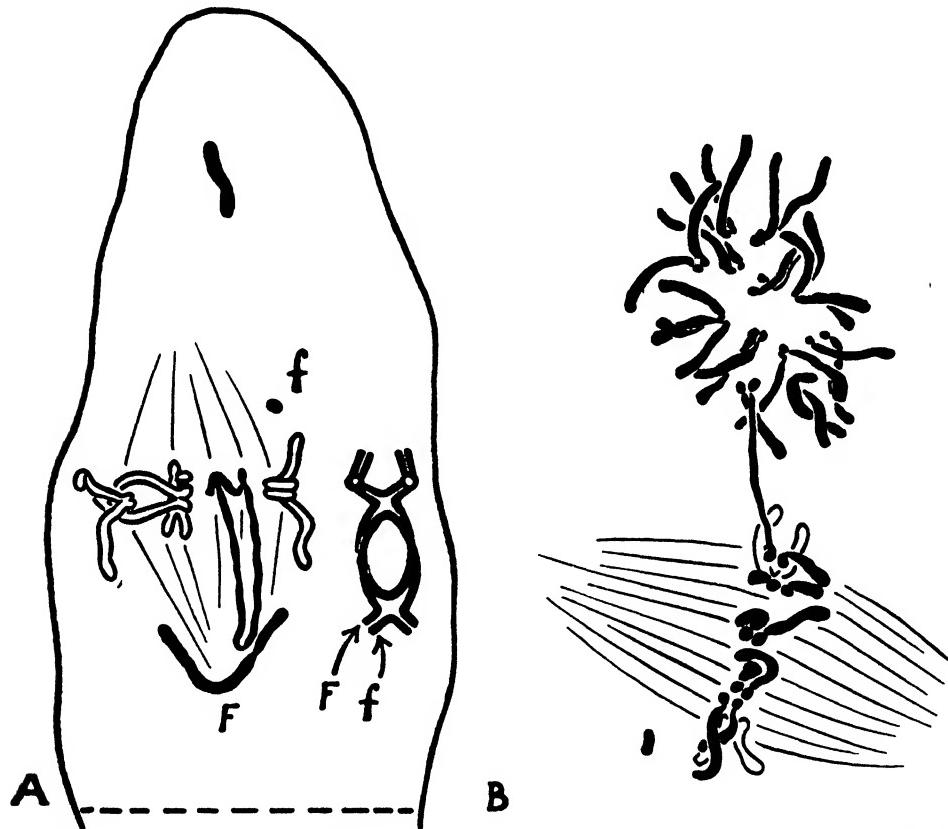
TEXT-FIG. 4. Second metaphases in *Lilium testaceum*. A, part of a restitution metaphase with 24 chromosomes. One shows a double first-division bridge of the type given in the inset diagram to which the large and small acentrics (*F* and *f*) also correspond. B, second metaphases whose position has been modified by a first-division bridge.

5. LATER DIVISIONS

Third divisions in the diploid *Fritillaria pudica*, *F. Meleagris*, *F. ruthenica*, and *F. latifolia* show the haploid number at the micropylar, and the triploid at the chalazal, end. Prefusion of the three chalazal nuclei has evidently taken place before metaphase, since the triple plate had a uniform arrangement.

At third telophase two large and two small nuclei were found in *F. Meleagris*, *F. Drenowskii*, and *F. pudica*.

Prefusion may be upset in some cases simply from a delay in the movement of one of the three nuclei to the chalazal end. Then two nuclei may fuse, as we

found in *F. Meleagris*, while two haploid nuclei are left at the generative end of the sac.

Another kind of upset probably arises from bridge formation. The two second metaphases can, as we saw, be held together by bridges. They are probably held closer than they would otherwise be. At second anaphase in
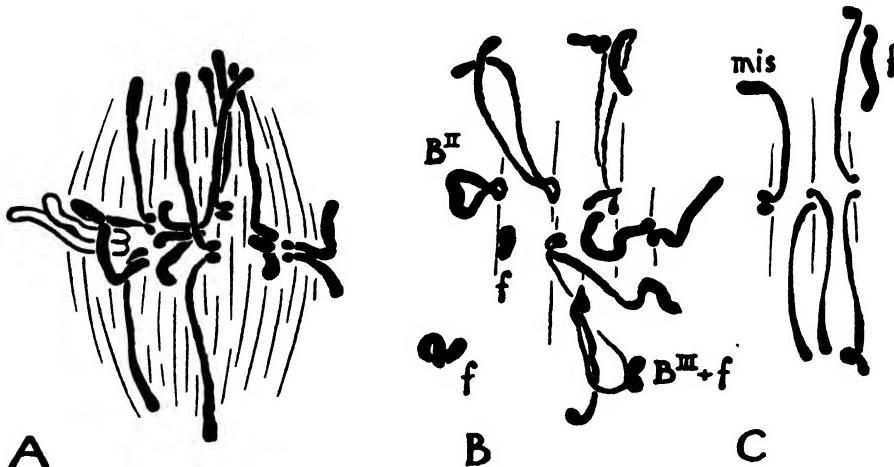
TEXT-FIG. 5. Parts of three second metaphases in *L. testaceum* showing stages in the stretching of the chromosomes by the spindle. A, a restitution metaphase. Note the touching of homologous ends of two chromosomes, which is only possible when the first division has failed. B, a second-division bridge (B^{II}), a non-disjunctional bridge (B^{III}), and three fragments, one adhering. C, two products of misdivision, three armed centric (*mis*), and one armed acentric (*f*).

L. testaceum the two groups of chromosomes moving towards the middle may then collide (Text-fig. 7 A) and form a diploid nucleus in the middle of the cell (Text-fig. 7 B). This might prevent any further prefusion, and at the third metaphase in *F. ruthenica* we have in fact found three plates—haploid, diploid, haploid.

The two large nuclei at the vegetative pole have been described as undergoing a fourth division parallel with the fourth division of the small nuclei at the generative end of the sac. This division, however, is characteristically delayed. Cooper speaks of it as abortive and Westfall more quaintly as 'amitotic'. Our observations of *F. Meleagris*, however, agree with their figures of *Lilium* in showing that the nucleus in fact degenerates before the fourth division. In this type of embryo-sac we have evidently the type of asymmetry that so commonly arises between the two poles of the embryo-sac in its later development. The first divisions are accurately synchronized. The later ones give either an advantage or, as in this case, a disadvantage to the vegetative pole.

The same kind of variable disadvantage arises in the ordinary Adoxa type

of three-division embryo-sac found in the Eriostemones tulips, which have no prefusion. Again it shows in the last division, which is one division earlier than in Fritillaria. In *T. primulina*, for example, we can see all four nuclei exactly synchronized or one of them slightly delayed (Pl. III, Figs. 11 and 13).
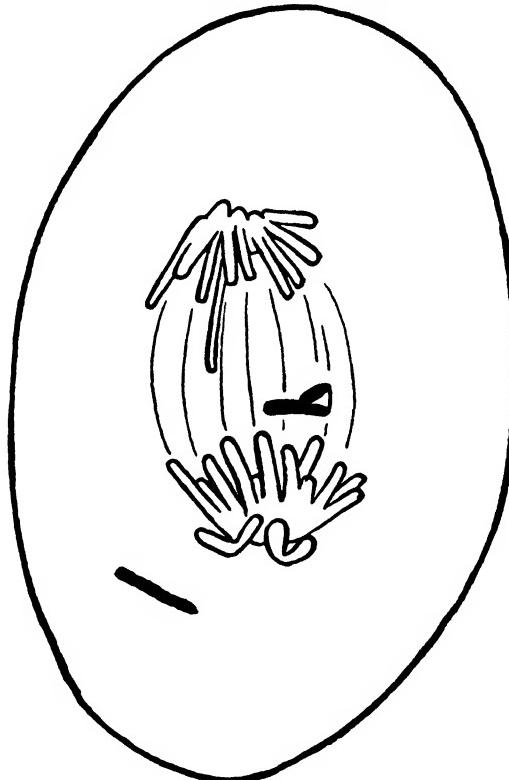
TEXT-FIG. 6. Misdivision of a daughter univalent and a telocentric product of misdivision at second anaphase in a *Lilium testaceum* embryo-sac.

In Fritillaria the products of the third division are genetically equivalent. In *Tulipa primulina*, although the products of the second division are not genetically equivalent, no cell wall has been formed between them. In both cases, therefore (see Section 7), we must assume that the delay is a position effect in differentiation like that occurring in the developing embryo.

6. CHROMOSOME ABERRATIONS OF THE MORPHOLOGICAL TYPE

Four important kinds of aberrations may now be regarded as likely to be characteristic of the Fritillaria type of embryo-sac development. This type, as Bambacioni-Mezzetti (1931), Cooper (1935), and Westfall (1940) have described it, may be represented as follows ($x = 12$, A, anaphase, P, prophase):

III P. 12 (12+12, 12) (polarization);

III A. 12, 12+36, 36 (prefusion);

IV A. 12, 12+12, 12+36, 36+36, 36 (maturity);

V P. $(24+12+12)+60+(36+36+36)$ (double fertilization).

Thus we have $2x$ embryos and $5x$ endosperm.

The aberrations will all affect nuclear movement and consequently fusion:
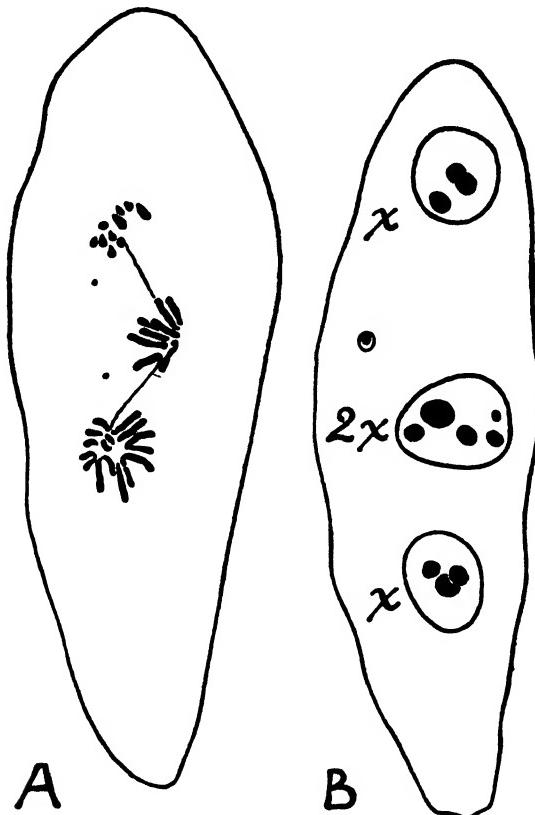
TEXT-FIG. 7. Fusion of middle nuclei at second anaphase, with bridges, in *Lilium testaceum*. Top group cut in A. ($\times 700$.)

(i) Polarity is reversed. Prefusion occurs at the chalazal instead of the micropylar end of the embryo-sac (Newton, 1927, *T. Kolpakowskiana*). Hence probably $4x$ embryos and $3x$ endosperm.

(ii) Polarity is superseded. Restitution nuclei are formed so that the ordinary arrangement in three and one is impossible (*L. testaceum*). Hence probably $3x$ embryos and $4x$ endosperm.

(iii) Prefusion is suppressed (Bambacioni, 1931, *Tulipa praecox*) or anticipated (*L. testaceum*). Hence probably $2x$ embryos and $3x$ or $4x$ endosperm.

(iv) Extra fusion of the chalazal nuclei gives hexaploidy (Cooper, 1935, *L. Henryi*). Hence probably $2x$ embryos and $7x$ or $8x$ endosperm.

One of these off-types has frequently been found in normal embryo-sac development, namely, the suppression of the first division and restitution

nucleus formation. There it is said to have the effect of turning the normal into the Allium type, since the embryo-sac contains the chromosomes of two spores (e.g. Nayaranaswami, 1940). Yet it makes a difference whether these nuclei are separate and haploid or fused and diploid in the derivative mitoses. The morphological description of a 'normal-type-with-restitution' as an Allium type disregards this distinction and relies on the number of nuclei instead of on their content. How misleading it is appears from the present Fritillaria type, where restitution has the same effect on meiosis and the chromosome content of nuclei as it has in the Allium type, although the tetrasporial embryo-sac remains tetrasporial.

Similarly we may recall the confusion that arose from morphologists supposing that four spores could be formed in diploid parthenogenesis. The absurdity of such an imaginary Alchemilla type has been pointed out by Gustafsson (1935).

All abnormalities of embryo-sac formation springing from failure of meiotic divisions must therefore be considered as morphologically of the type with normal meiosis from which they are derived. The *maternal* type is prior to the *filial* accident.

A fifth abnormality, bridge formation, is of even more general importance. It has been described in the embryo-sac of *L. tigrinum* (Westfall, 1940) and we may infer its occurrence from Capoor's illustration of Urginea (1937). The first division bridges are of the most account because their supposed behaviour has been used to explain the non-occurrence of inversion crossovers (or corresponding inviable progeny) in Drosophila (Sturtevant and Beadle, 1936). The tension on the bridges pulls their pairs of centromeres towards one another and thus controls their orientation. They will divide in such a way that the dicentric chromatids will fall to the two middle nuclei and not to an end one. They thus cannot enter the egg nucleus in Drosophila. The drosophilists' conjecture is vindicated by the behaviour of the lily.

It should be noted, however, that this effect will not hold good for second-division bridges. Such bridges arise where crossing-over occurs proximal to the inversion as well as within it. The absence of recovered inversion crossovers from female Drosophila is thus to be expected only when the inversion extends to a point close to the centromere or interferes with crossing-over between it and the centromere.

The effects of bridge formation on the second-division movements of the chromosomes are clear. Their effects on the later fusion of the nuclei are also, as we saw, important in Lilium and Tulipa where the four nuclei are linear and prefusion occurs.

In other species of Tulipa without prefusion results will be different. The linear arrangement of the four nuclei is not maintained, and we do not know whether the one of them which is to be the egg is predetermined by its relative position in the sac. Differentiation may, as we saw, show in *T. primulina* itself in the precocious rate of development of the third prophase in three of

the four nuclei, which by this time lie in a square. Bridge formation will no doubt modify this arrangement and so affect the determination of the egg.

7. GENETIC VERSUS SPATIAL DIFFERENTIATION

In monosporial embryo-sacs and uninucleate pollen grains the differentiation that occurs between chalazal and micropylar cells or between vegetative and generative cells is one of spatial organization like that in the development of an animal or plant embryo. On the other hand, the differentiation or competition between dissimilar spores in Oenothera depends on a genetic difference.

Now this genetic differentiation seems to be possible only when an effective cell-wall is formed between the dissimilar nuclei, for Barber (1941 *a, b*) finds that when such a wall is not formed in a binucleate pollen grain a deficient nucleus (provided it contains several chromosomes) will continue to develop at the same rate as a balanced nucleus. The question then arises as to whether this is a general principle. Does it apply to the early tetrasporial embryo-sac which has no partitions or can genetic differences act to modify the rate and kind of development of its several and dissimilar unpartitioned nuclei in the way we have seen? At a later stage, of course, cell-walls are developed and the differences found in the relative development of haploid and diploid cells from one embryo-sac to another in Pyrethrum (Martinoli, 1939) (unlike those between similar triploid nuclei in Fritillaria) must be attributed to their genetic differences. Similarly, if cell-walls are the means of rendering genetic differentiation effective we must look upon the temporary walls of Aeonium and Leontodon in the potentially monosporial type, and of Peperomia in the regularly tetrasporial type, as being adaptive rather than merely accidental (cf. Bergman, 1935; Maheshwari, 1937).

Here then is a physiological problem. The varying interactions of chromosomes, nuclei, and cells in the development of the embryo-sac can be studied and compared, not only in their different systematic groups but in their different interlocking genetic consequences, consequences which in different hybrids and polyploids provide a model experiment in the relations of nucleus and cytoplasm.

8. SUMMARY

1. Twin embryo-sac mother cells occur in *Tulipa Gesneriana*.

2. Cap nucleoli are found at diplotene in the embryo-sac but not in the pollen mother cells of several species of Fritillaria.

3. The same degree of localization of chiasmata is found in embryo-sacs as in pollen mother cells in eleven species of Fritillaria.

4. The spindle develops more slowly at the first and second metaphases in the large embryo-sac than in the small pollen mother cell of Lilium and Fritillaria. Hence, perhaps there is a greater stretching of the parts of the chromosomes lying within it.

5. Inversion crossing-over leads to bridge formation at first and second divisions in Lilium crosses and Tulipa triploids. The bridges modify the shape of the spindle and the orientation of second-division chromosomes in a way that is not possible in the confined space of the pollen mother cell. This will produce the Sturtevant and Beadle effect on inversion crossing-over in egg formation.

6. Misdivision of univalents is found at first and second anaphase in the cross *Lilium testaceum*.

7. Restitution nuclei are found in *L. testaceum* in the embryo-sac although not in the pollen mother cell. They should suppress the polarity of the embryo-sac and modify the endosperm as well as the embryo.

8. The major spiral is maintained in some parts of the chromosomes and lost in others at second metaphase in Tulipa and Fritillaria. This unstable equilibrium is never found at the first division.

9. Evidence of triple prefusion was found after the second division in four species of Fritillaria.

10. The development of an embryo-sac is influenced by genetic segregation and by mechanical irregularities at meiosis. Hence these must be considered in assigning it to a morphological type and interpreting variability of behaviour. Hence also it seems likely, *inter alia*, that the Renner effect can occur in all flowering plants which are sufficiently hybrid to make its occurrence important.

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EXPLANATION OF PLATES VI & VII

Illustrating the article by Dr. C. D. Darlington and Mr. L. La Cour on 'The Genetics of Embryo-sac Development'.

PLATE VI

Figs. 1–3. First metaphase in the embryo-sac of *Lilium testaceum* with univalents and a reduced chiasma formation. (× 1,200.)

Figs. 4 and 5. First metaphases in *Fritillaria involucrata* and *F. pallidiflora* with localization of chiasmata partial and absent. (× 600.)

Figs. 6 and 7. Second metaphase at two focuses in a triploid tulip (Text-fig. 3). (× 1,200.)

Figs. 8 and 9. First anaphase bridges in *L. testaceum* and *Tulipa lanata* (3x). The first shows the bridge pulling the spindle askew. The second shows the third member of a trivalent disjoining. (× 1,200.)

PLATE VII

Fig. 10. Twin mother cells in a garden tulip seedling; second metaphase shows in one of them. (× 600.)

Fig. 11. Third anaphase in two of the four nuclei of *Tulipa primulina*. (× 1,200.)

Fig. 12. Chalazal fusion nucleus at anaphase in *Fritillaria pudica* ($3x = 36$). (× 1,200.)

Fig. 13. Third prophase in *T. primulina* showing retardation of one of the four nuclei. (× 1,800.)
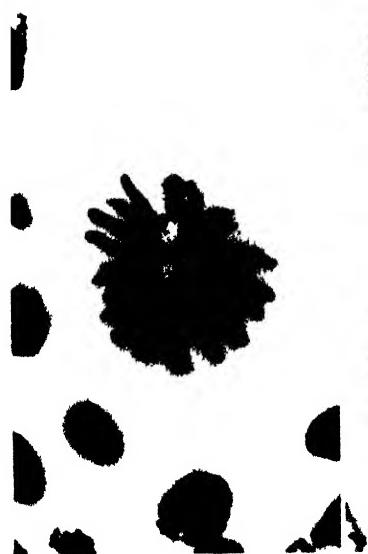
On the Morphology of the Pitcher-Leaves in Heliamphora, Sarracenia, Darlingtonia, Cephalotus, and Nepenthes

BY

AGNES ARBER

With five Figures in the Text

1. INTRODUCTION

THOUGH much has been written on the subject, a certain obscurity still surrounds the morphology of pitcher-plants. In the present paper an attempt is made to approach the interpretation of the pitcher from a somewhat different standpoint from that generally adopted; the aim has been to understand the morphology through a study of the relation of the parts making up each kind of pitcher, and through a comparison of those of different genera, rather than through an attempt to fit these peculiar phyllomes into the conventional framework of the typical foliage-leaf. After a brief description of those features in the pitchers which seem to be of significance from this point of view, their comparative morphology will be considered in the discussion.

Troll (1939, pp. 1852–6, and 1866–97) has recently given a comprehensive account of pitcher-plants, with a documented analysis of the opinions which have been held at various times upon their foliar morphology. It is thus unnecessary to go over the same ground here, so the references to the literature will be merely incidental.

Most of the pitcher-plants are rare and difficult to obtain, and I am greatly indebted to those who have given me specimens for study; to Professor D. H. Campbell, Stanford University, California, and to the Director of the Royal Botanic Gardens, Kew, for Darlingtonia; to the Regius Keeper of the Royal Botanic Garden, Edinburgh, for Cephalotus and Heliamphora; to Professor

HELIAMPHORA NUTANS Benth.
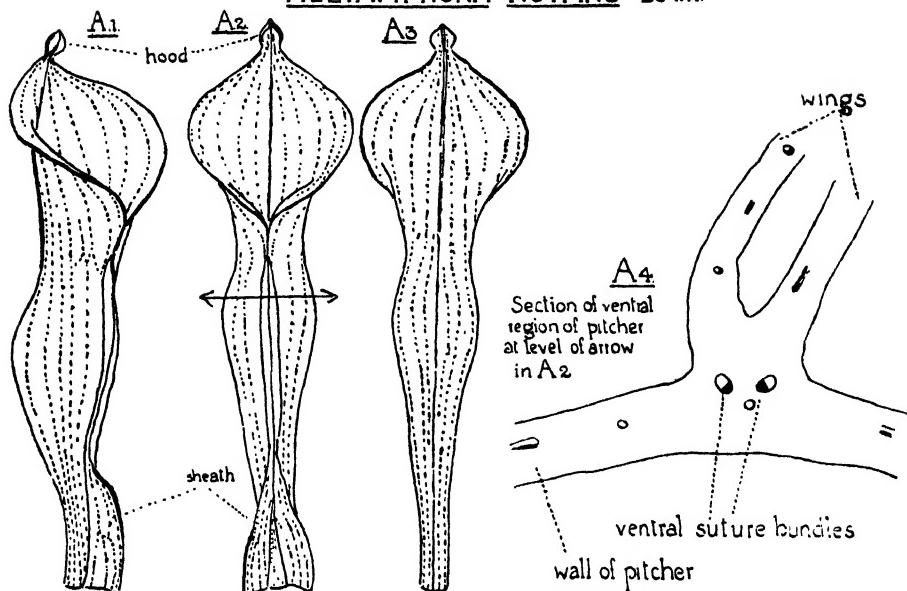
SARRACENIA PURPUREA L
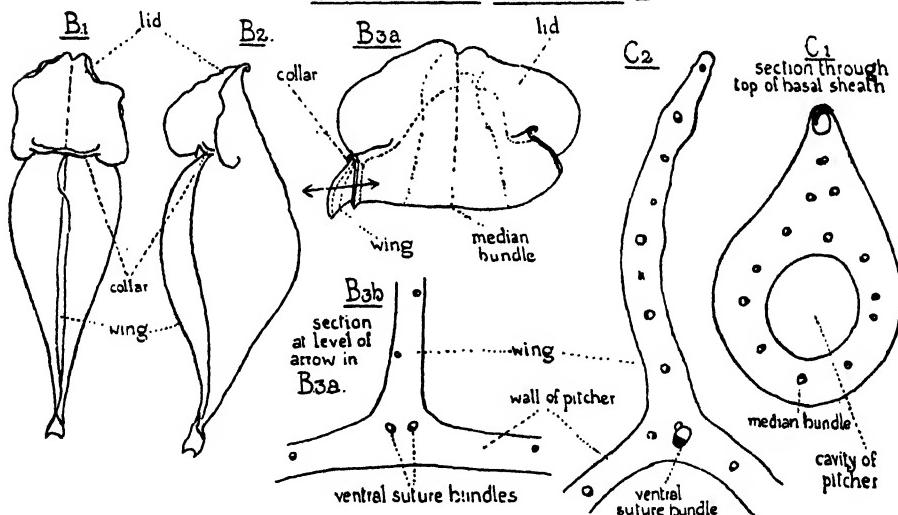
FIG. 1. (Throughout the text-figures xylem is shown black, phloem white, and fibres dotted.) A, *Heliamphora nutans* Benth., Roy. Bot. Gard., Edinburgh, Aug. 27, 1940. A1, side view; A2, adaxial view; A3, abaxial view of a pitcher ($\times \frac{1}{2}$). Bundles seen in surface view indicated by dotted lines. In A2 the sheath is opened out more widely than in the natural position. A4, transverse section of junction of wings and tube at approximately the level of the arrow in A2 ($\times 14$). B and c, *Sarracenia purpurea* L., Camb. Bot. Gard., Jan. 5, 1922. B1, adaxial view; B2, side view, of pitcher ($\times \frac{1}{2}$). B3 *a*, top of the same pitcher spread out after cutting open at the side of the suture. B3 *b*, transverse section ($\times 14$) at level of arrow in B3 *a*, passing through junction of wing and pitcher wall. c, transverse sections from another

G. E. Nicholls, the University of Western Australia, for Cephalotus; and to the Director of the Cambridge Botanic Garden, for Sarracenia.

2. Description

(i) *Heliamphora* (*Fig. 1*, A)

The horn-like pitcher or tube of *Heliamphora nutans* Benth. is of a relatively simple type; the cavity is carried down into the base of the phyllome, and no region which can be called a petiole is differentiated. The principal veins take a parallel course, and ultimately converge towards the midrib. The sheath-wings (Fig. 1, A_1, A_2) are, as usual, developed from the region of intersection of the adaxial and abaxial faces of the leaf. At the top of the sheath these two boundary regions make contact, so that the adaxial surface vanishes; they remain in touch up to the base of the aperture, where they part company. The two junction-lines between the adaxial and abaxial faces of the phyllome thus form the right and left margins of the aperture. From the leaf-base to a little below the aperture, these two junction-lines are winged. There are two main ventral bundles, one corresponding to each wing (Fig. 1, A_4). The upper surface of the leaf-base, and the surfaces of the sheath-wings and tube-wings which are turned towards one another, and also the inner lining of the tube, all belong to the adaxial face of the leaf, while the exterior of the tube, and the under surfaces of the wings, belong to the lower (abaxial) face of the leaf. The leaf apex is very slightly hooded. The margin of the pitcher mouth shows a minimal trace of a roll-over to the outside, not sufficiently pronounced to be called a collar. The sides of the little terminal hood are formed in part by the continuation and hypertrophy of this rim.

(ii) *Sarracenia* (*Fig. 1*, B, C)

The pitcher of Sarracenia is similar to that of Heliamphora, but in its form it diverges still further from a typical foliage-leaf (Fig. 1, B_1, B_2). As in Heliamphora, the invagination penetrates downwards into the leaf-base region (Fig. 1, C_1). Whereas paired wings, associated with two ventral bundles, arise along the ventral line of the Heliamphora tube, in Sarracenia there is a single wing, showing no external sign of doubleness, and supplied by a single series of bundles only. These bundles, however, face in two opposite directions, thus revealing the dual origin of the wing (Fig. 1, B_3 *b*, C_2). There is a single ventral suture bundle (Fig. 1, C_2), but below the aperture this strand divides into two, which form the marginal bundles of the rim. It thus seems best to regard the single suture bundle as representing two united ventral bundles, which separate below the aperture. The rim of

pitcher ($\times$ 14). C_1, near top of basal sheath to show that the invagination penetrates into the leaf-base region. C_2, through the wing at a higher level, showing the bundles with xylem facing to right and left.

DARLINGTONIA CALIFORNICA Torr.
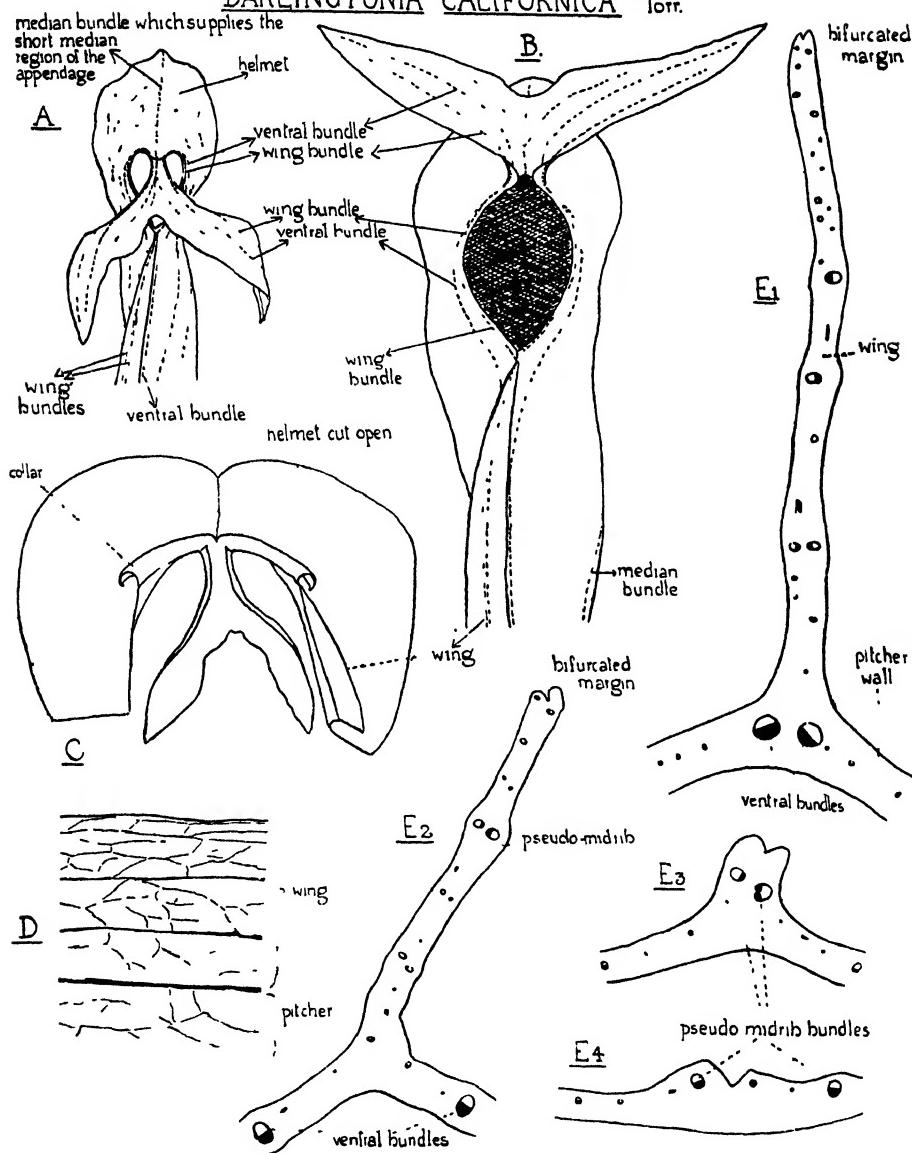
FIG. 2. *Darlingtonia californica* Torr., Roy. Bot. Gard., Kew, 1940. A, adaxial view of helmet (× ½). B, adaxial view of helmet with the fish-tail appendage turned up in an unnatural position, and its turned-over margin spread out flat except at the base. On a larger scale than A, and slightly diagrammatized to show the principal veins more clearly; other veins omitted. C, helmet of another pitcher slit open dorsally along midrib, and ventrally beside wing; seen from within. This sketch shows that the margins of the appendage are continuous with the collar of the pitcher (on a slightly larger scale than A). D, small segment of wing (× about 3½), with its junction with the pitcher, to show that the venation is primarily longitudinal. E1–E4, series of transverse sections (× 14) from below upwards through the wing; these drawings

the aperture has a definite, external, curve-over collar. A considerable part of the dorsal margin of the aperture grows up into a lid- or hood-like appendage. The form and venation indicate that the lateral parts of the lid represent a localized expansion of the rim of the aperture on either side of the midrib (Fig. 1, B3 *a*).

A noticeable feature of the pitcher venation in Sarracenia is that there is a convergence towards the midrib apex both of the main longitudinal strands, and also of the horizontal bundles (derived from the ventral bundle) which supply the collar (Fig. 1, B3 *a*).

(iii) *Darlingtonia (Fig. 2)*

The pitcher of *Darlingtonia californica* Torr. resembles that of Sarracenia in general construction, but differs from it in being conspicuously helmeted in its distal region. Moreover, the lid of Sarracenia is represented in Darlingtonia by an apical fish-tail appendage, which droops over the mouth opening (Fig. 2, A). The two wings of the leaf-base are carried up jointly into a ventral wing. This wing appears at a casual glance to be single, but it is seen on closer inspection to have a grooved margin, while in anatomy it distinctly shows its duplex character. The wing emerges between two suture bundles (Fig. 2, E1), and within the wing there are two distinct series of strands, while at certain levels there is a pseudo-midrib consisting of two bundles facing one another. In the upper region of the wing, the tendency to bifurcation at the margin becomes more marked, and at the apex of the wing this double edge opens out to form the turned-in roll-collar which borders the aperture. If the helmet is cut open so that the collar can be followed round towards the midrib, it is found that the collar, when it approaches the dorsal line, grows outwards to form the two lobes of the fish-tail appendage (Fig. 2, C). The relatively short median part of the appendage is a continuation of the midrib region of the leaf. This analysis of the fish-tail appendage is confirmed by the venation. The two bundles of the pseudo-midrib of the wing separate below the aperture of the pitcher (Fig. 2, E1–E4), and run round its margin until they reach the base of the fish-tail appendage. They enter it and each pursues a course near one of its outer edges (Fig. 2, B). Similarly the ventral (suture) bundles of the pitcher, which have passed upwards near the line of origin of the wing, move apart at some distance below the aperture, and take a course more or less parallel to that of the wing bundles. At the top of the aperture the ventral and wing bundles approach one another closely, though remaining

are from hand sections, so the relation of the individual bundles in the different sections cannot be followed accurately. E1, at about 13 cm. from the top of the helmet, and more than 18 cm. from the base of the tube. E2, about 1·5 cm. from the base of the helmet aperture. E3, a little below, and E4, just below, base of helmet aperture. The two main bundles which reach the top of the wing are separating asymmetrically above the wing. In E3 and E4 the ventral bundles have separated so widely that they are not included in the part of the section drawn.

CEPHALOTUS FOLLICULARIS Labill.

A.1

A.2a

lid with venation, ribs, & "windows" indicated

A.3

main lateral dorsal vein

lateral wing

thickened area in interior of pitcher (dotted)

median flange & wings

main lateral dorsal vein

median bundle

winged median flange

A.2.b.
section of two minor ribs from lid

rib

bundles with xylem pointing downwards

"window"

A.2.c.
segment between the arrows in A2b.

A.4

lid

lower edge of cornice

A.5.

lid

main lateral hook

dotted lines show position of external attachment of lateral wings

collar & cornice

main lateral hook
lateral wing
median hook

thickened area (dotted)

winged median flange

winged median flange

FIG. 3. *Cephalotus follicularis* Labill. A1, A2 *a*, A3–A5, Roy. Bot. Gard., Edinburgh, Aug. 1940; A2 *b* and A2 *c*, King George's Sound, Western Australia, 1922. A1, side, and A2 *a*, back view of a pitcher (×½); principal veins shown; in A2 *a* the lid is raised up, and its venation ribbing, and 'windows' are indicated. A2 *b*, transverse section of part of a lid of another pitcher showing two minor ribs and a 'window' between them (×14). A2 *c*, the part of A2 *b* between the arrows (×77 circa). A3, adaxial view of pitcher, veins omitted (×½). A4, pitcher seen from above with the lid bent backwards, veins omitted (on a slightly larger scale than A3). A5, pitcher cut down beside the median flange and opened out (×½ circa); the dotted lines show position of external insertion of lateral wings.

distinct. They then move apart again, the ventral bundles, like the wing bundles, entering the appendage, each running into one of the lobes. The apical end of the median bundle, with its branches, occupies the relatively inconspicuous median region of the appendage.

(iv) *Cephalotus (Figs. 3 and 4)*

Cephalotus follicularis Labill. bears both typical foliage-leaves (Fig. 4, F) and also pitcher-leaves (Fig. 3, A1–A3). The latter differ from those already described in possessing a well marked petiole intercalated between the leaf-base and the pitcher; its attachment to the pitcher is not basal, but is situated slightly below the distal aperture (Fig. 3, A1). In the area of attachment, the margins of the adaxial face of the petiole form two wings or flanges, delimiting the corresponding adaxial surface of the pitcher; this surface passes into that of the lid (Fig. 3, A2 *a*). The xylem of the lid bundles is directed downwards.

The rim of the pitcher aperture has a conspicuous collar with incurved teeth or hooks (Fig. 3, A4). Three teeth, more highly developed than their neighbours, are set respectively above the median flange and the two dorsal-lateral wings, with which we shall deal shortly. On cutting the pitcher open, it is seen that, below the collar, and running completely round the throat of the aperture, there is an internal projection, which may be termed a cornice. The relation of the cornice to the hooked part of the collar will be understood from Fig. 3, A5, where the rim of the pitcher is seen in longitudinal section.

The median bundle of the phyllome, leaving the top of the petiole abaxially, passes down the back of the pitcher to its base, and then turns up the front, running near the external margin of the median flange (Fig. 3, A1, A2 *a*). This flange is winged to right and left for most of its length, but, towards the apex the wings narrow and disappear, and the flange ends above in a free point.

Besides the wings marking the boundary between the adaxial and abaxial surfaces of the pitcher, and the wings associated with the midrib, there is another pair of wings occupying a lateral-dorsal position. The lowest points of the external pitcher surface which these wings reach, coincide in position almost exactly with the apices of two thickened areas which occur on the *inside* of the pitcher wall (cf. Fig. 3, A1 and A5). The main dorsal-lateral bundles of the pitcher descend from the level of attachment of the petiole until they approach the lowest points of the lateral wings. They then branch, and one branch of each curves upwards, and runs towards the top of the pitcher in the lateral wing, while the other, after reaching a point about half-way down the thickened area, turns upwards, and then takes a course more or less parallel to the other branch (Fig. 3, A1).

Up to this point the present description may be looked upon as a confirmation of the clear account given by Dickson sixty years ago; but we must now turn to the ventral bundle system, to which he did not pay detailed attention.
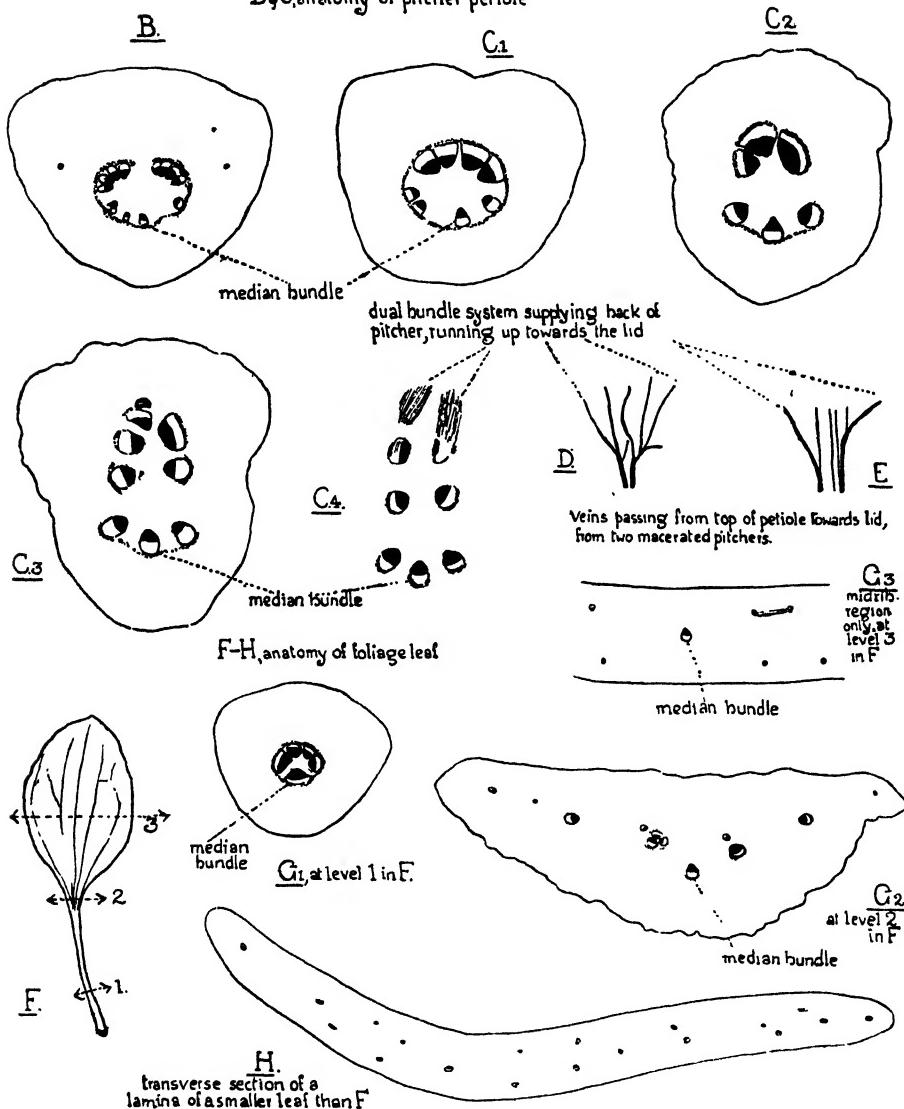
FIG. 4. *Cephalotus follicularis* Labill. B, D, E, H, King George's Sound, Western Australia, 1922; C, F, G, Roy. Bot. Gard., Edinburgh, August 1940. B, transverse section low in pitcher-petiole ($\times$ 14). C1–C4, sections ($\times$ 14) from a series from another pitcher-petiole, from below upwards. Since these are hand sections, the individual bundles cannot be followed accurately. C1, which is higher in the petiole than B, is about $\frac{3}{4}$ in. below pitcher; C2, about $\frac{1}{4}$ in. below pitcher; C3, very near attachment to pitcher; C4, bundles only, from extreme top of petiole, showing bundles passing into the back of the pitcher, and running up towards the lid. D and E, from macerated preparations of two pitchers to show veins passing up from the adaxial region of the petiole apex towards the lid (slightly enlarged). F, foliage leaf ($\times \frac{1}{2}$). G1–G3, transverse

For understanding this system maceration is useful. I have found that, for material which has been preserved successively in spirit and in formalin solution, 'Milton' at full strength is a convenient reagent for rendering the pitcher soft and translucent, and making it easy to dissect out the veins. It is possible by this means to see that the ventral part of the vascular system, which passes up from the adaxial face of the petiole, mainly to supply the lid, is more or less symmetrical about two principal veins (Fig. 4, D and E). It thus takes the form which the anatomy of the petiole would lead one to expect. Fig. 4, C1–C4 show that in the petiole the ventral bundles, on either side of the median line, tend to retain their distinctness, so that the system which passes into the adaxial region of the pitcher is duplex (Fig. 4, C4). Fig. 4, B (which is from a different petiole from the C series, and cut at a lower level) indicates the twofold character of the ventral bundle system with special clearness. The form and the scheme of venation of the lid are consistent with the dual symmetry of its vascular supply (Fig. 3, A2 *a*). The lid has thickened ribs, with translucent 'windows' between them (Fig. 3, A2 *b* and A2 *c*), the thickening being so arranged that there is no median rib. The associated vascular tissue also shows poor development in the median as compared with the lateral regions. This tendency to a dual symmetry seems to have been overlooked by Troll in his full account of Cephalotus, but in his fine photographs of the pitchers, the absence of a median rib in the lid is very obvious (1939, Fig. 1590, p. 1854); Hamilton also noticed this point in 1904.

(v) *Nepenthes* (*Fig. 5*)

The pitcher-leaf of Nepenthes (Fig. 5, A1) recalls that of the genera already described, but with certain marked differences. The basal region is much prolonged, and has highly developed laminar wings (Fig. 5, A1, A4, B1–B3); it is separated from the pitcher by a slender stalk which may be called descriptively a petiole.

Not only the basal, but also the distal region of the Nepenthes pitcherphyllome is peculiar among pitcher-plants. The aperture is edged by a double collar, with an inward and an outward curve-over, both of which are vascular (Fig. 5, D). At the back of the aperture there are two members—a lid on the adaxial side, and, abaxially, a little pointed structure, which may bear small lateral branches, and is generally bent somewhat backwards (cf. Fig. 5, A1–A3). In the young pitcher the lid is depressed so as to close the aperture completely and firmly (Fig. 5, C1, C2, C4, E1). The xylems of the lid bundles are turned downwards (Fig. 5, C3). The median point shows a certain tendency to radial anatomy, but the bundles, in the species which I have examined, are orientated irregularly, and the structure is not neatly unifacial (Fig. 5, E2 and F).

sections of leaf F ($\times$ 14). H, transverse sections of a lamina of another smaller foliage leaf, to show relative unimportance of midrib ($\times$ 14).

NEPENTHES
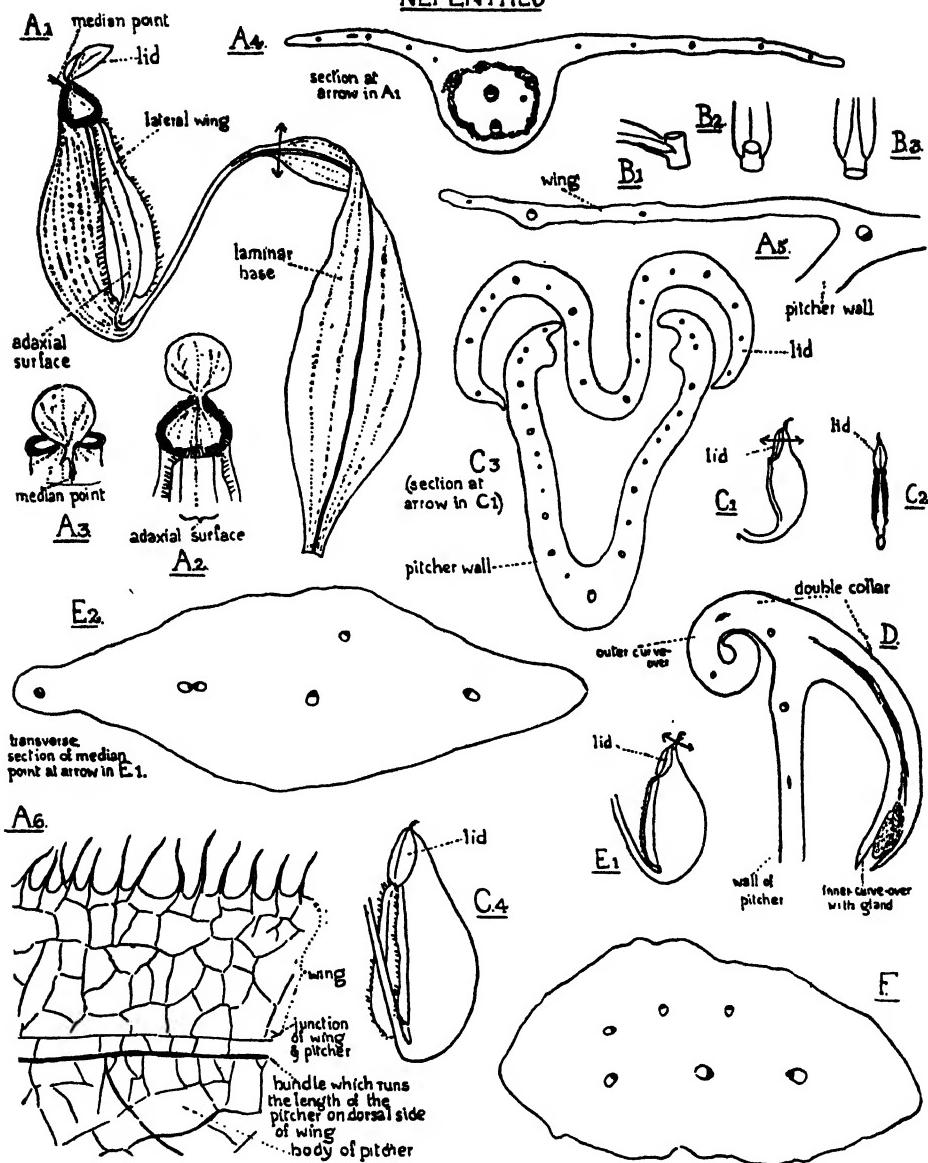
FIG. 5. Nepenthes. A1–A6, *N.sp.*, Cambridge Bot. Gard., Aug. 15, 1940. A1, leaf ($\times \frac{1}{2}$), not quite complete to base. A2, front view, A3, back view (from above) of top of pitcher, on a slightly larger scale than A1. A4, transverse section near apex of laminar base, at arrow in A1 ($\times$14). A5, transverse section of upper part of one of the wings bordering the adaxial strip ($\times$23); the bundles have xylem above and phloem below. A6, small segment of wing from pitcher drawn in A1 ($\times$about 3$\frac{1}{2}$). B1–B3, base ($\times \frac{1}{2}$) of a leaf of *N. albo-cincta* var. *rubra*, Camb. Bot. Gard., Jan. 4, 1922. B1, side view; B2, adaxial view; B3, abaxial view. C and D, *N. Williamsii* Masters, Camb. Bot. Gard., July 18, 1940. C1, side view, and C2, adaxial view of a young pitcher ($\times \frac{1}{2}$). C3, transverse section in neighbourhood of arrow in C1 ($\times$14). D, longi-

3. DISCUSSION

Studies on the ontogeny of Sarracenia, Darlingtonia, Cephalotus, and Nepenthes, have shown that the tube or pitcher is the distal part of a phyllome modified by an invagination which involves the apical end of the adaxial surface (Hooker, 1859; Eichler, 1881; Goebel, 1891). The pitcher is thus lined by the adaxial (upper) face of the phyllome, which also forms the lower surface of the lid, hood, or appendage. Examination of the mature structure in Heliamphora, Darlingtonia, and Sarracenia, shows that the wings are in contact, or in union with one another along the ventral line of the leaf, and thus the adaxial surface is reduced to the point of disappearance, so far as the pitcher exterior is concerned. In Heliamphora the wings, which are associated with two distinct ventral bundles (Fig. 1, A4), are in contact along their bases only, whereas in Darlingtonia and Sarracenia they unite into one structure. In Darlingtonia the wing, though it seems simple on casual inspection, does, in fact, show its duplex character both externally and anatomically (Fig. 2, E1–E4). The main rib of the wing consists at certain levels of two bundles facing one another, recalling the pseudo-midrib of the equitant leaf in some of the Iridaceae, e.g. Tritonia (Arber, 1918, p. 483, Fig. 15, C; or 1925, p. 62, Fig. xxxviii. 15, C), or of such a phyllode as that of *Acacia uncinella* Benth. (Arber, 1921, p. 318, Fig. 42; or 1925, p. 105, Fig. lxxix. 42). In Sarracenia the union of the wings has become more intimate than in Darlingtonia. In place of the two ventral bundles of Darlingtonia, each of which corresponds to one wing (Fig. 2, E1), we find in Sarracenia a single suture bundle (Fig. 1, C2), which only separates into two at a high level of the wing (Fig. 1, B3 *b*). Macfarlane (1889) noted the resemblance between the wing of Sarracenia and an Iris leaf; this resemblance can be traced in detail in the anatomical scheme (e.g. compare Fig. 1, C2, and Fig. 2, E1, with Arber, 1918, p. 483, Fig. 17B; or 1925, p. 62, Fig. xxxviii. 17B).

Nepenthes and Cephalotus differ from the three genera just considered in the fact that the adaxial surface of the outside of the phyllome is not narrowed out of existence, but is represented in the body of the pitcher, though on a relatively small scale. In Nepenthes this adaxial surface is a wing-bordered strip, which runs the length of the pitcher (Fig. 5, A1, A2). In Cephalotus, on the other hand, the adaxial surface is not strip-like, but forms, as Dickson pointed out (1881), a wedge-shaped area, passing upwards from the top of the petiole, and outlined to right and left by narrow wings; the area in question is dotted in Fig. 3, A2 *a*. In addition to the wings bounding the adaxial surface, the pitcher of Cephalotus has two other pairs of wings. One of these pairs arises from the median flange associated with the midrib, which runs up

tudinal section of top of pitcher wall, and of the collar which surrounds the aperture ($\times$ 14). E, *N. Hibertii*, Camb. Bot. Gard., Sept. 23, 1940. E1, young pitcher ($\times \frac{1}{2}$); E2, transverse section of median point at arrow in E1 ($\times$ 47). F, *N. coccinea*, Camb. Bot. Gard., Sept. 23, 1940. Transverse section of the median point of a young pitcher ($\times$ 47).

the dorsal face of the pitcher. According to Dickson, the apical point of this flange, being terminal to the midrib, may be held to represent the leaf apex. The third pair of wings is associated with the lateral-dorsal veins. They belong entirely to the abaxial surface, and may be regarded as forming 'secondary' margins to the phyllome, comparable with those described by Troll for certain petioles (1931, pp. 368, 9; 1939, p. 1211, Fig. 981, III).

It is perhaps doubtful whether the word 'wing', which we have used for the longitudinal outgrowths borne by the pitchers in the five genera here considered, is always the best term. It is possible that those of Heliamphora, Darlingtonia, and Sarracenia are better described as 'borders', for they have a definite longitudinal venation like the rest of the pitcher (cf. Fig. 2, D), and like the basal 'laminar' part of the Nepenthes leaf (Fig. 5, A1). The outgrowths of the Nepenthes pitcher, on the other hand, have an essentially reticulate venation (Fig. 5, A5), and here the word 'wing' seems appropriate.

The five types of pitcher-plant which are under review show varying degrees of elaboration in the rim structure of the apical aperture. In Heliamphora there is a very slight indication of an outward roll-over, while in Sarracenia a similar, but more developed outer curve-over, forms a distinct collar. In Darlingtonia there is also a simple roll-collar, which curves inwards. Cephalotus has a complex toothed collar, curving inwards, with a cornice-like inner development below, while Nepenthes is peculiar among these genera in having a double collar with an inward and an outward curve-over.

The top of the pitcher in each of these five genera is not only bordered by a collar, but it bears also a further outgrowth: the hood of Heliamphora; the lid-flap of Sarracenia; the fish-tail appendage of Darlingtonia; the lid of Cephalotus; the lid and median point of Nepenthes. The nature of these outgrowths has always been one of the moot questions in discussions about the morphology of the pitcher, which hitherto seem invariably to have been based on the attempt to correlate the outgrowths with parts of an ordinary foliage-leaf—a procedure leading inevitably to a series of strained explanations. In the present paper a fresh interpretation of a less ambitious kind is offered, based upon the effort to understand the pitchers *in themselves*, rather than in relation to the foliage-leaf, which is an arbitrary choice among phyllome forms. The idea I wish to suggest is that *the outgrowths are due to the inter-action of two structural features—collar development and general venation*. In Heliamphora it is the continuation of the rudimentary curve-over of the pitcher lip which forms, at least in part, the sides of the very small median hood, while in Sarracenia the fact that the lid is merely a localized development of the collar and median region is more evident. In Darlingtonia the continuity of the collar with the marginal lobes of the fish-tail appendage can readily be traced, while it can be seen that the stunted median region of the appendage is supplied by the median vein. The pitcher top is thus formed on a corresponding plan in the three genera, *the upgrowth from the margin being a localized over-development of the elsewhere narrow collar*. It is conceivable that

this over-development may be due to hypertrophy in the median region associated with the parallel venation of the pitcher, which involves convergence of veins towards the midrib at the apex, so that a disproportionate supply of food material may accumulate in this neighbourhood.

In Cephalotus the lid may again be interpreted as a hypertrophy of the collar region. That the lid is essentially of the same nature as the collar is indicated by the fact that the cornice continues unaltered below both the collar and the lid. It is possible that the thickened ribs of the expanded lid are equivalent to the hooks of the collar. An important difference between Cephalotus and the other genera considered is that the lid in the former is ventral instead of dorsal. It is thus not the convergence of the veins towards the midrib which is, in this case, correlated with the lid expansion, but rather the relatively high development of the ventral system of the pitcher venation. This unusual vascular development on the ventral face can be traced in the petiole also. It should be explained that Troll's account of the anatomy of the petiole does not conform exactly to the structure in the material which I have been able to study. He states that the stalk of the pitcher-leaf has unifacial anatomy, with a ventral median bundle, which he figures (1939, p. 1855, Fig. 1591, I, and p. 1856). This is important from his point of view, since he regards peltation as a *consequence* of unifacial petiole structure, and he interprets the pitcher of Cephalotus on the same lines as a peltate leaf. In my specimens, however, as is shown in Fig. 4, B–E, the ventral bundles do not fuse, so that the petiole structure cannot be called unifacial in Troll's sense. It is true, however, that in the petiole of the pitcher, as well as in the petiole and even the lamina of the foliage leaf, the bundles are not uniseriate as in typical foliage leaves (Fig. 4, G1–G3, H).

There has been much divergence of opinion about the interpretation of the distal part of the Nepenthes pitcher with its two members at the back of the aperture—the median point, and the lid (cf. Fig. 5, A1–A3). The earlier writers (cf. Candolle, 1827) were inclined to regard the lid as representing the whole lamina, while theories of the later nineteenth century (Bower, 1889, and Macfarlane, 1889) treated it as a compound structure, arising from the 'congenital coalescence' or 'exaggerated dorsal fusion', of two leaflets in front of the leaf apex. According to the most recent opinion, on the other hand (Troll, 1939), the lid is the *Querfieder* (transversal pinna) developed on the adaxial side of a peltate leaf. Though so many views have been suggested, it seems doubtful whether any one of them is really an adequate interpretation. If the lid is taken to represent a complete lamina, it is impossible to see how to explain the rest of the phyllome in terms consistent with this. On the other hand, Bower and Macfarlane's idea that the lid represents two fused leaflets, is too complex an hypothesis to accept without more cogent evidence than has been adduced. It is true that the lid is sometimes slightly indented at the tip, and that the main lateral veins tend to be stronger than the median one; but the predominance of the marginal over the median region is not uncommon

in leaf members the singleness of which is unquestioned. Against the third view—Troll's interpretation of the lid as a *Querfieder*—there is one piece of evidence which seems decisive: if the lid is of this nature, the xylems of the bundles should be directed upwards (i.e. to the side towards the median point, which he regards as the leaf apex), whereas in fact they are orientated in the opposite sense (Fig. 5, C3). In support of his hypothesis Troll lays stress on what he describes as the unifacial character of the anatomy of the median point, and he figures a transverse section of the point in *Nepenthes alata*, as showing bundles with their xylems all facing inwards (1939, Fig. 1629, IV, p. 1897). In *N. coccinea* (Fig. 5, F) and in *N. Hibertii* (Fig. 5, E2), however, I have noticed that though the bundles tend towards a radial arrangement, their orientation is not of the regular unifacial type which Troll describes for *N. alata*. Since unifacial structure thus proves not to be a universal character of the median point, it can scarcely be used to support Troll's interpretation. So, as there seems to be some reason for discarding each of the ingenious hypotheses just enumerated, it will be best to give up, for the time being, the attempt to describe the Nepenthes pitcher-phyllome in terms of a typical foliage-leaf, and to concentrate on getting a clear conception of the pitcher-leaf considered *in itself*. I have come to think that this may be best achieved by envisaging the lid and median point of Nepenthes on the same lines as have been followed in the present paper in picturing the relations of the out-growths in other pitcher-plants. Now the pitcher mouth of Nepenthes, as we have already noted, is unique among the genera described in having a double collar—a curved-over vascular outgrowth on the inner side, and a similar, less highly developed outgrowth, also vascular, on the outer side (Fig. 5, D). The view which I wish to propose is that both the lid and median point are merely localized expressions of the collar-forming activity which is responsible for the double curve-over of the aperture edge—the lid, which is turned down in youth, corresponding to the inner curve-over, and the median point to the outer curve-over. The relative hypertrophy of the lid and median point may be correlated with the special characters of the venation, which, as in the other pitchers considered, is of the parallel type. The midrib passes directly to the junction region of lid and median point, while the veins of the abaxial part of the pitcher (i.e. of the whole pitcher except the narrow adaxial strip) also show a strong tendency to converge upon the apical region (Fig. 5, A1–A3). The median point and the lid can thus draw upon a richer vascular supply than the rest of the aperture collar, which is entered only by minor lateral veins, and thus localized overgrowth in the median region may be stimulated.

Although according to the view here suggested, the lids of both Cephalotus and Nepenthes are alike in corresponding to the inner turn-over of the pitcher collar, they differ materially in that the lid of Nepenthes is a dorsal member, with a single median bundle, while that of Cephalotus is a ventral member with two main bundles. It is true that the pitcher lids of these two genera have a certain general similarity in appearance and relations. More-

over, they both recall the laminae in certain foliage-leaves; but so, for instance, does such a member as the distal element of the ligular sheath in Eichhornia, which no one would think of homologizing, in a strict morphological sense, with an ordinary leaf lamina (Arber, 1922, Pl. I, Fig. 8; or 1925, p. 108, Fig. lxxxi, 8). Such laminar forms are interesting, not as homologues of one another, but as examples of one of those *Gestalt* types, occurring repetitively in the flowering plants, which Troll has been the pioneer in distinguishing and analysing (cf. Arber, 1937). Since these types may be built up of varying structural elements, they cannot be treated as homologous; but since they attain the same goal, though by a diversity of means, they fall into one category as expressions of the same *Gestalt* tendency.

A noticeable feature of all the pitcher-leaves considered is the important part played in the venation by the ventral or marginal bundles in comparison with the midrib. This feature is conspicuous, for instance, in Darlingtonia, in which, as we have seen, the lobes of the fish-tail appendage, which are supplied by the marginal bundles (ventral and wing), much exceed in length the median region supplied by the midrib. This feature illustrates a general trend—the tendency for main 'axes' to become subordinated to their own laterals—which has recently been discussed elsewhere (Arber, 1941).

4. Summary

An attempt is made to interpret the morphology of the pitcher-leaf in Heliamphora, Sarracenia, Darlingtonia, Cephalotus, and Nepenthes. The pitcher-leaf of each genus is considered, firstly, in itself and, secondly, in comparison with the pitchers of the other genera, rather than with typical foliage-leaves.

The chief problem to be faced in considering pitcher-leaves is the nature of the outgrowths from the distal aperture, i.e. the hood of Heliamphora, the lid-flap of Sarracenia, the fish-tail appendage of Darlingtonia, the lid of Cephalotus, and the lid and median point of Nepenthes. The view here put forward is that *these outgrowths may all be interpreted, on corresponding lines, as a localized hypertrophy of the collar bordering the pitcher aperture.* This interpretation is confirmed by the fact that Nepenthes, the only genus which has at the apex both an inner and outer outgrowth (the lid, and the median point), is also the only genus which has both an inner and an outer collar. It is suggested that *the hypertrophy, which produces the lids and other appendages of these pitcher-plants, is conditioned in each case by a special localized richness in vascular supply.* In Heliamphora, Sarracenia, Darlingtonia, and Nepenthes, the parallel veins of the pitcher converge towards the midrib at the apex, and it is from this strongly vascular median region that the lid or other appendage is formed. In Cephalotus, on the other hand, the lid is a ventral member, but here again we find that it occurs where there is a peculiarly strong apparatus of bundles, for the pitcher-petiole is unusual in having a more pronounced

vascular supply towards the adaxial face than in the midrib region, and it is these adaxial bundles which enter the lid.

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The Cytology of Gaulthettya wisleyensis (Marchant) Rehder
A New Mode of Species Formation

BY

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With four Figures in the Text

THE ericaceous plant *Gaulthettya wisleyensis* (Marchant) Rehder was described recently by Mulligan* (1939). Produced at Wisley, it was thought on morphological grounds to be a natural hybrid between the North American species *Gaultheria Shallon* Pursh. and the South American species *Pernettya mucronata* (Linn. f.) Sprengel. At the suggestion of Sir Arthur Hill, Director of the Royal Botanic Gardens, Kew, I examined the chromosomes of Gaulthettya, *Gaultheria Shallon*, and *Pernettya mucronata* in order to check the conclusions reached on morphological grounds. Chromosome counts of certain other species of Ericaceae were also made, and these have been included in the list of chromosome numbers of the Ericaceae given in the table (p. 5).

Mitotic counts were taken from sections of root-tips fixed in La Cour's 2 BE and stained by Newton's gentian violet-iodine method (La Cour, 1937). The maturation divisions of pollen mother cells of Gaulthettya, *Gaultheria Shallon*, and *Pernettya mucronata* were examined by means of the same technique.

With one exception, the *Gaultheria-Pernettya* complex has a basic chromosome number of 11. *Gaultheria Itoana* is the exception in having a diploid number of 26 (Fig. 4 *d*). This may represent an ancestral condition in this group, since 13 is the basic number of the Ledeae, Rhododendreae, and Arbuteae groups of the Ericaceae.

Gaultheria Shallon is octoploid with 88 chromosomes. I cannot confirm Hagerup's count of 96 (Hagerup 1928). *Pernettya mucronata* is hexaploid with 66 chromosomes. *Gaulthettya* is heptaploid, and this tallies with the view that the two former plants are its parents (44+33 = 77) (Fig. 1). *Pernettya furiens*, with which Gaulthettya has sometimes been confused, is hexaploid.

Both *Gaultheria Shallon* and *Pernettya mucronata* seem to be autopolyploids, since high multivalent associations occur in both species (Fig. 2, *a* and *b*). Thus associations as high as octavalents are found in *Gaultheria Shallon* and hexavalents in *Pernettya mucronata*.

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* The plant was named *Gaulnettya* × Wisley Pearl by Mulligan, but this was a *nomen nudum*. Rehder (Manual of Cultivated Trees and Shrubs, 2nd edition, 1940) gave it the name *Gaulthettya wisleyensis* and provided a brief English description.

In Gaulthettya there is a great deal of physiological sterility on the male side: in many anthers the pollen mother cells reach the first meiotic metaphase, but
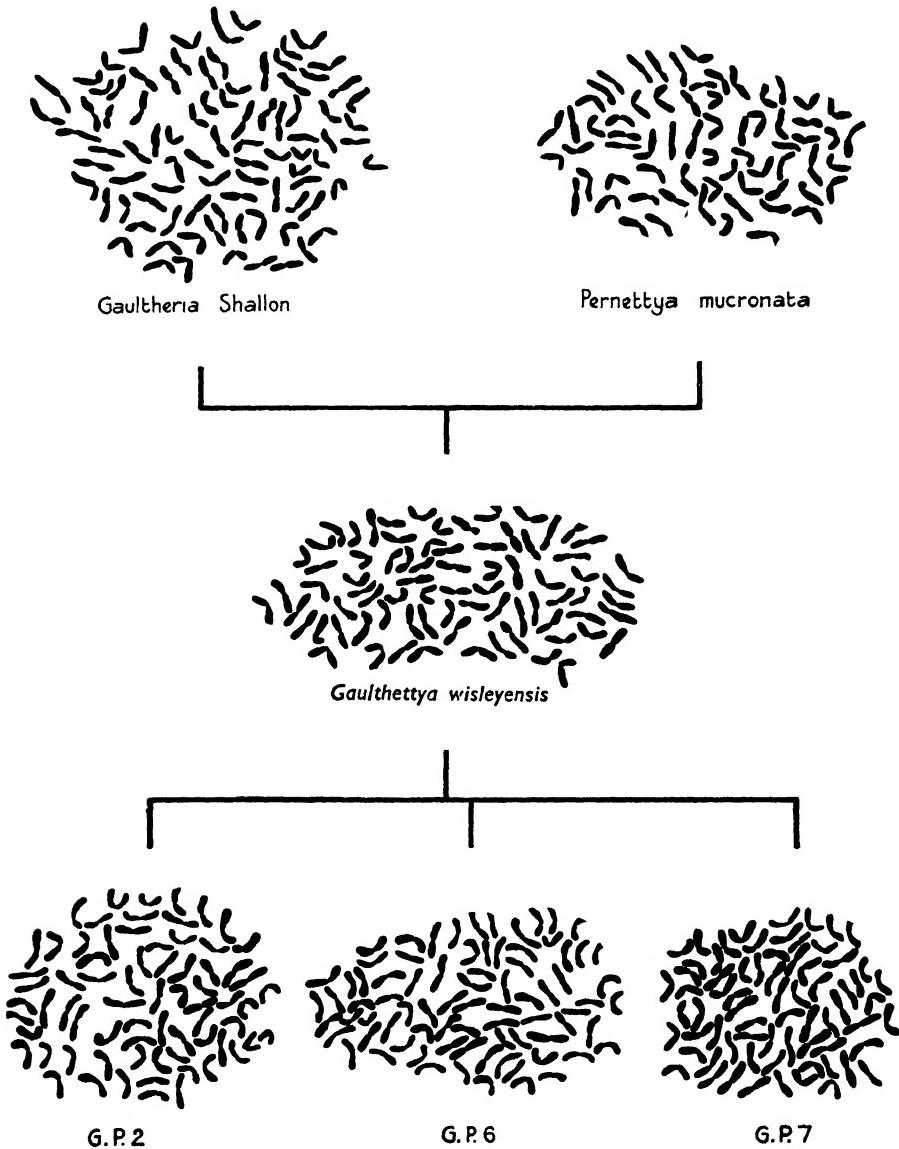
FIG. 1. Metaphase plates from root-tips illustrating relationships of *Gaultheria Shallon* (2n = 88), *Pernettya mucronata* (2n = 66), and *Gaulthettya wisleyensis* (2n = 77). G.P. 2, 2n. = 71. G.P. 6, 2n = 79. G.P. 7, 2n = 70. (×3,500.)

anaphase does not follow and they degenerate at this stage. In the degenerating cells the spindle is intensely stained in gentian violet preparations; the chromosomes appear to liquefy and coalesce. This is a condition of the

anther as a whole; other anthers show no degeneration of the pollen mother cells at first metaphase.

In general between five and ten univalents are found at each first metaphase (Fig. 2c). This stage therefore shows far less failure of pairing than is typical
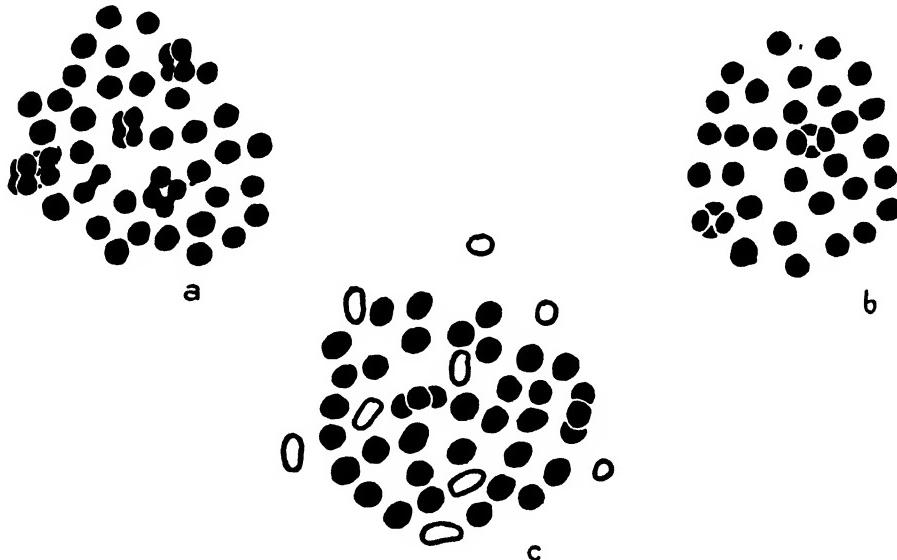
FIG. 2 *a–c*. Polar views, first meiotic metaphase. *a. Gaultheria Shallon*, 32II + 6IV. *b. Pernettya mucronata*, 29II + 2IV. *c. Gaulthettya wisleyensis.* 9I + 3III + 2III. (× 7,000.)

of a newly arisen hybrid springing directly from diploid parents. Pairing in such a hybrid is mainly conditioned by the degree of homology existing between the haploid complement of one parent and that of the other. In the case of Gaulthettya, however, we must bear in mind that its probable parents are autopolyploids. Such pairing as takes place is therefore probably pairing within the reduced complements of each parent. Gaulthettya effectively consists of a tetraploid Gaultheria component added to a triploid Pernettya component. The failure of pairing which does exist in Gaulthettya probably depends on its Pernettya component; trivalents do not form regularly because of a low chiasma frequency per chromosome.

The constitution of Gaulthettya approximates to that of an allopolyploid hybrid; its relatively regular pairing is a parallel function. Its origin, however, is to be contrasted with that of an allopolyploid. In an allopolyploid, identity of chromosome partners arises by the doubling of the diploid hybrid, e.g. *Primula kewensis* and *Raphano-Brassica*. In Gaulthettya, on the other hand, the doubling preceded hybridization (Fig. 3).

Although less fertile than either of the parent species, about 10 per cent. of the open-pollinated seeds of Gaulthettya are full. Both Mulligan and I have raised seedlings showing genetic segregation. Mine were from isolated plants
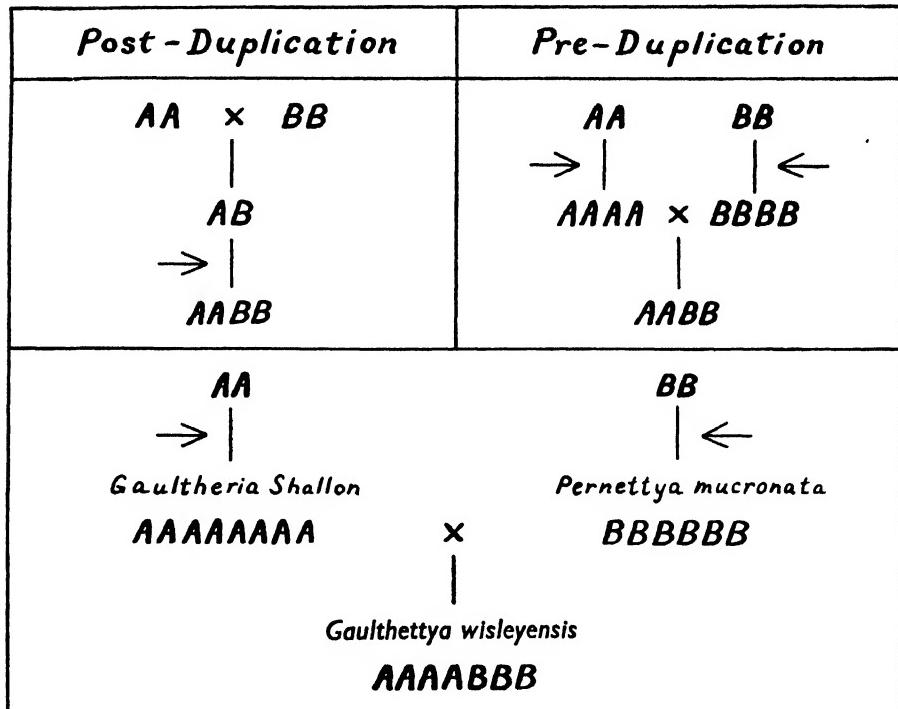
FIG. 3. Diagram to illustrate the relationship between allopolyploidy (post-duplication) and the polyploidy of Gaulthettya which has arisen by pre-duplication.
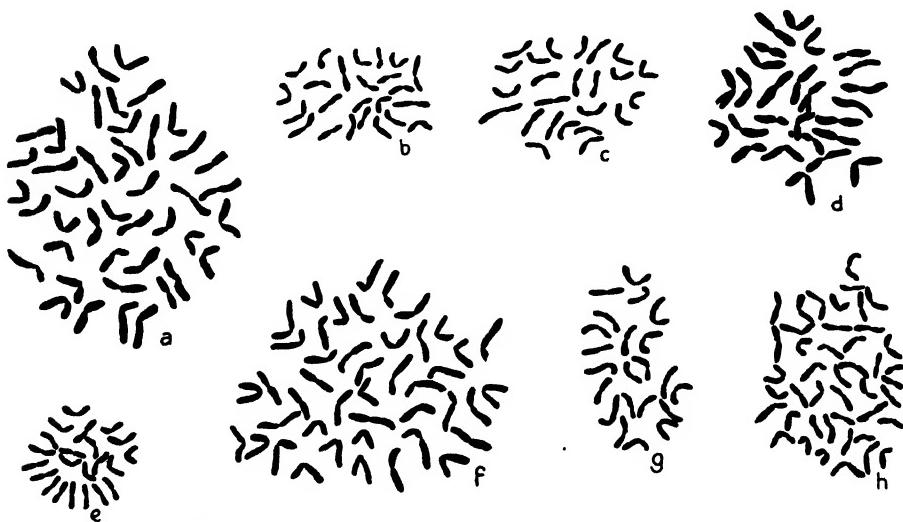
FIG. 4 *a–h*. Metaphase plates from root-tips of certain Ericaceae. *a. Kalmia polifolia*, $2n = 44$; *b. Leucothoe acuminata*, $2n = 24$; *c. Pieris Mariana*, $2n = 24$; *d. Gaultheria Itoana*, $2n = 26$. *e. G. cuneata*, $2n = 22$. *f. G. Cumingiana*, $2n = 44$. *g. Pernettya tasmanica*, $2n = 22$; *h. P. pentlandii*, $2n = 44$ ($\times 3,500$.)

presumably selfed; three of these, G.P. 2, G.P. 6, and G.P. 7, have been examined and they are all aneuploid, with 71, 79, and 70 chromosomes respectively (Fig. 1). The viability of these aneuploid plants depends on their being high polyploids; a few odd chromosomes disturb their genetic balance less than if they were lower polyploids.

The fertility of G.P. 1 and G.P. 2 is very poor. I raised one seedling from G.P. 2, but this plant died six months after germination, being then only 1 cm. high. Fertile new species could only arise from Gaulthettya if by chance a seedling arose with a balanced and paired mixture of Gaultheria and Pernettya chromosomes. This is not an impossibility, especially since the odd chromosomes could be lost in stages through a number of generations. Physiological sterility would probably act as a greater barrier to this form of speciation than meiotic irregularity due to numerical unbalance of the chromosome complement concerned. Provided there were little physiological sterility, a fertile hybrid could, however, arise direct from two autotetraploid parents, from two auto-octoploid parents, or from one autotetraploid and one auto-octoploid parent.

The cultivated strawberry is a comparable example, it being the fertile octoploid hybrid between two octoploid species, *Fragaria chiloensis* and *F. virginiana* (Darlington, 1932).

Hybridization within and between the two genera Gaultheria and Pernettya has occurred very commonly in the wild state (Burtt and Hill, 1935). It is probable that some speciation in this complex and elsewhere has occurred by the hybridization of autopolyploid species.

SUMMARY

Cytological evidence supports the view that *Gaulthettya wisleyensis* is a hybrid between *Gaultheria Shallon* and *Pernettya mucronata*. Gaulthettya is heptaploid ($2n = 77$), *Gaultheria Shallon* is octoploid ($2n = 88$), and *Pernettya mucronata* hexaploid ($2n = 66$).

The fertility of *Gaulthettya* depends on the autopolyploid nature of its parents. Hybridization of autopolyploids may be an important mode of speciation.

Chromosome Numbers ($2n$) in the Ericaceae

RHODODENDROIDEAE

LEDEAE

Ledum groenlandicum	26 Hagerup (1928), Wanscher (1933)
„ *columbianum*	26 Callan

RHODODENDREAE

Rhododendron many spp.	26 Sax (1930) and others
,, *calendulaceum* ⎫	
,, *canadense* ⎭	52 Sax (1930)

PHYLLODOCEAE

Leiophyllum buxifolium	24 Hagerup, 1928
Loiseleuria procumbens	24 ,, ,,
Kalmia latifolia	24 ,, ,,
,, *glauca*	48 ,, ,,
,, *polifolia*	44 Callan
Phyllodoce coerulea	24 Wanscher (1933)
Daboecia cantabrica	24 Maude (1940)

ARBUTOIDEAE

ANDROMEDEAE

Cassiope hypnoides	48? Hagerup, 1928
Leucothoe acuminata	24 Callan
Andromeda polifolia	48 Hagerup, 1928
Pieris Mariana ⎫	
,, *Forrestii* ⎪	
,, *lucida* ⎬	24 Callan
,, *japonica* ⎭	

GAULTHERIEAE

Gaultheria Itoana	26 Callan
,, *hispida* ⎫	
,, *cuneata* ⎪	
,, *antipoda* ⎬	22 Callan
,, *glomerata* ⎭	
,, *Cumingiana* ⎫	
,, *Griffithiana* ⎬	44 Callan
,, *Shallon*	96 Hagerup (1928), 88 Callan
Pernettya tasmanica	22 Callan
,, *prostrata* ⎫	
,, *pentlandii* ⎪	
,, *ciliata* ⎬	44 Callan
,, *buxifolia* ⎭	
,, *mucronata* ⎫	
,, *furiens* ⎬	66 Callan

ARBUTEAE

Arbutus 2 spp.	26 Hagerup, 1928
,, *arachnoides* ⎫	
,, *xalapensis* ⎭	26 Callan
Arctostaphylos, 2 spp.	26 Hagerup, 1928
,, *bicolor* ⎫	
,, *pungens* ⎭	26 Callan

VACCINIOIDEAE

VACCINEAE

Gaylussacia baccata	24 Longley (1927)
Vaccinium, 5 spp.	24 ,, ,, , Hagerup (1928)
,, *uliginosum*	24, 48 Hagerup (1928)
,, 3 spp.	48 Longley (1927)
,, 3 spp.	72 Longley (1927), Hagerup (1928)

THIBAUDEAE
    Pentapterygium serpens   24 Callan

ERICOIDEAE
    Calluna vulgaris   16 Hagerup (1928), Hahn, 1929 (Tischler, 1931)
    Erica, 6 spp.   24 Hagerup (1928), Maude (1940), Wanscher (1933)

    E. curvirostris
    E. sessiflora   }   24 Callan
    E. Willmoreana
    Bruckenthalia spiculiflora   36 Hagerup (1928), Callan

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The Genus Calvaria, with an Account of the Stony Endocarp and Germination of the Seed and Description of a New Species

BY

ARTHUR W. HILL

With twenty-six Figures in the Text

CALVARIA MAJOR (vernacular name Tambalocoque) is a tree found only in Mauritius; it is also known as 'Bois de Natte', though other native trees appear to bear the same name. The trunk has large spreading buttresses at the base, which commence some 4 ft. above ground-level, the edges like the trunk being smooth and rounded (Vaughan and Wiehe, 1937, 1941). It is found in the tropical Upper Montane rain forest from 1,500 to 2,600 ft. elevation, where the rainfall is from 100 to 200 in. The timber is very hard and heavy and is used by shipwrights for stem and stern curves and is resistant to termites.

The Acting Conservator of Forests also informs me that it is one of the slow-growing, indigenous trees which are dying off in the upland forest, due possibly to the invasion of exotic weeds whose dense undergrowth prevents the regeneration of the timber trees. Another factor which may be causing the dying off of these trees is the depredation of monkeys, also exotic, who destroy the fruits, though what use they can be to them, except as missiles, it is hard to see!

Seeds, therefore, are only available from trees in private gardens and natural regeneration has not been observed in the forests.

Like other endemic trees of the island Calvaria has no doubt suffered from the increase of the sugar plantations, which resulted in large areas of native forest being felled between the years 1810 and 1875, and also owing to the indifference of the Government and Forest Department in the past towards the native flora. The Guardian of Woods and Forests wrote in his Annual Report for 1887: 'Introduced kinds grow best in the warmest districts . . . so that the disappearance of the Mauritian kinds . . . is a matter of no regret. . . . Naturalists complain (or rather would-be Naturalists) that the indigenous vegetation is disappearing and being replaced by foreign plants which they know naught of . . . the sooner the greater part of the indigenous trees which are left disappear, the better will it be for all concerned' (Vaughan and Wiehe, 1937, p. 291). It is no wonder that Thompson, when he visited the island in 1880 to report on the forests, found them 'a picture of doleful ruin' (Thompson, R., 1880).

The genus Calvaria was founded by Commerson on a one-seeded, apple-like fruit—to which the persistent calyx was still attached—the thick, woody seed lying horizontally in the fruit. Commerson, however, did not publish his generic name, though he figured the fruit in his inedited drawings, which are probably at Paris, but it is to Gaertner (1805), that we owe our knowledge of this remarkable fruit. Gaertner's figures do not represent very clearly the full significance of Commerson's generic name, nor of his specific epithet *cerebellina*, which it is presumed refers to the embryo contained in the 'brain pan' or 'skull', but the close resemblance of the smooth, upper surface of the large woody endocarp to the top of a human skull is very marked in the more recent material which has been received at Kew from Mauritius, and fully justifies the generic name.

Gaertner describes and figures the fruits or seeds of three species: (1) *C. major*, to which he refers Commerson's *C. cerebellina* ined. with a query; (2) *C. hexangularis*; and (3) *C. globosa*. The seed specimen of his *C. major* he states is in the collection of seeds in the Paris Museum, while those of the other two are in the Deléssert collection, but unfortunately nothing is said as to their country of origin.

Engler (1890) at the end of his account of the Sapotaceae includes Calvaria and refers to the seed lying horizontally, stating that the three insufficiently known species come from Madagascar.

In his monograph of the Sapotaceae (1904), however, he includes *Sideroxylon grandiflorum* (= *C. major*) and gives Mauritius as its locality, but does not refer to Gaertner's two other species.

He includes one other species with *S. grandiflorum* in his section Calvaria of the genus Sideroxylon, namely *S. imbricarioides* A.DC., the 'Bois de fer' from the Island of Réunion. The type of *S. imbricarioides* A.DC. is Commerson's *S. laurifolium* in the Paris Herbarium. The species is also represented at Berlin by a specimen in Kunth's Herbarium labelled ' De Neumanno emtum, 1825', determined by Engler as *S. imbricarioides* A.DC. (1904).[1] When De Candolle described this species (1844) he did so on vegetative and floral characters only and assigned it to 'Bourbon or Mauritius', but Cordemoy (1895), who gives a full description of the tree, cites the Island of Réunion only as its locality. He describes it as a large tree with buttresses, bearing fruits the size of a small lady-apple—'pomme d'api'—(? Siberian Crab). The single seed very hard and attached at the base by a large hilum, which occupies at least the lower third of the seed. The young flowers of the Berlin plant closely resemble those of *Calvaria major* and *C. galeata* (Fig. 20), and the ovary bears a ring of hairs at the base. The seed appears to be a small edition of that of

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[1] The Berlin specimen consists of a stem with leaves and young flowers only and the label reads 'Insula Franciae vel Borboniae', though Engler (quoting Neumann 1825), gives Réunion as the locality.

The Berlin specimen may possibly be a duplicate of Commerson's type, for Kunth had many dealings with Paris and often bought specimens from Neumann, who otherwise is unknown.

C. major, the large hilum described by Cordemoy being no doubt the lower unpolished half of the seed (see Fig. 5).

Though he makes no mention of the upper part of the seed or of its horizontal position in the fruit, it may be inferred, from his account of the fruit of his section Calvaria—in which he places this species—and from his reference to Baillon's figures, that the seed is horizontal and that *Sideroxylon imbricarioides* is a true Calvaria. Though unfortunately no specimen of the fruit has been seen, the description of the tree so closely resembles *C. major* in all particulars that I have no hesitation in assigning it to that genus. The correct citation therefore becomes **Calvaria imbricarioides** (*A. DC.*) *A. W. Hill*.

Reverting to the three species figured by Gaertner. The fruit of *C. major* he describes as 'a fleshy, ovate berry, exceeding a goose's egg in thickness, but a little shorter, conically attenuated upwards from a very thick base, obtuse, turbinate'. The large, single, subglobose seed is 'differentiated into two hemispheres, the upper being depressed and unequally ovate, exactly recalling the shape of a skull, very smooth from a little above the middle, rusty-bay-coloured and terminated by an acute margin, with the lower part obtusely umbonate, narrower, scabrous, consisting of a wide boss-shaped area, which is continuous with the upper hemisphere, and marked with unequal pits—usually 3 in number—on the boss'.

The seed of *C. hexangularis* is said to be rather larger than the nut of a hazel—'Semen nuce avellana paullo majus'—depressed and obscurely six-angled, elliptic. The figure of the upper surface of the seed shows a smooth, elliptic structure marked by six, slightly convex, triangular lobes, separated by six slight depressions meeting at a point at the centre of the upper surface.

The seed of *C. globosa* is similar to that of *C. hexangularis* and is described as being rather larger than that of a bean (faba), depressed and angularly elliptic.

In all three cases the seed is said to be horizontal, and is so figured, and the embryo lies horizontally in the seed cavity. In general form the seeds both in *C. hexangularis* and *C. globosa* resemble the much larger seed of *C. major* in having the lower portion of the stony endocarp quite different from the upper polished part. They are also somewhat smaller in diameter, with a roughened or corrugated surface marked with pits, which were the points of attachment of the vascular strands.

In the Kew Herbarium, material is preserved of four fruiting specimens of Calvaria from the islands of Mauritius and Rodriguez. The specimens are as follows:

1. *C. major* consisting of specimens with leaves, flowers, fruits and seeds, which have recently been augmented by a fine collection of young and mature fruits, kindly sent by the Forest Officer, Mauritius (Figs. 3–11).

2. Two fruit stones .. '. dug up in Mauritius from the Marsh whence Dodo bones are obtained ... found lying with the bones'. These were sent over in 1893, at the request of Sir Edward Newton, who wished to know if they belonged to anything the Dodos were likely to have eaten. The fruit stones of

C. major especially are remarkably like the stone preserved in the Zoological Museum, Cambridge, both in size and shape, which according to the account in the 'Encycl. Britt.' were swallowed by the Dodo and were used by the Dutch sailors for sharpening their knives. This stone is figured by Newton and Clark in 'Phil. Trans. Roy. Soc.' 1879, vol. clxviii, pp. 449 et seq., plate 47, figs. 4 and 5. The stone in the Cambridge Museum, however, comes from the gizzard of the 'Solitaire' which inhabited Rodriguez. It may well be that the stony 'seeds' of Calvaria were used by these peculiar birds as well as the stones of fine volcanic rock. They closely resemble the 'stones' of *C. globosa* figured by Gaertner and may be referable to that species.

3. A leafy specimen from Mauritius collected by Dr. Philip B. Ayres of the Mauritius Civil Service, received in February 1864 with a fruit cut in half in a capsule fixed to the sheet. The half fruit still retains its calyx.

4. A recent specimen from Rodriguez with fruits and seeds, collected by Mr. H. C. King of the Forestry Service, brought over and presented to Kew by Dr. R. E. Vaughan, and another, bearing flowers and leaves (vernacular name 'Bois de Pomme'), collected by Mr. R. Jauffret at Cascade Victoria, Rodriguez, also presented to Kew by Dr. R. E. Vaughan. These prove to represent an undescribed species, which is distinguished from the others in having the seed or stone erect in the ovoid fruit instead of lying horizontally (see Figs. 12–19, 21–22).

The only other specimen from Rodriguez at Kew is one consisting of leaves and stem only, collected by Dr. I. Bayley Balfour on the Transit of Venus Expedition in 1874. This has rather smaller leaves than Mr. King's specimen, but the indumentum is similar and no doubt it belongs to this species.

In the 'Flora of Mauritius', Baker (1877) refers *Calvaria major* Gaertn. f. to *Sideroxylon grandiflorum* A.DC., which is based on specimens collected by Bojer and Bouton in Mauritius, the type of which is preserved at Kew.

Four other species of Sideroxylon are described by Baker from Mauritius, but all of them, though they closely resemble *C. major* in their leaves and flowers, have small fruits about the size of a pea and are quite unlike those of Calvaria.

Hemsley (1897) wisely restored Calvaria as a genus and says, 'it is probably as distinct a genus as many others in the order'. Had he been able to study the fruits more carefully and observe their mode of germination, he would, I think, have regarded Calvaria as a peculiar genus probably of great antiquity and far removed from any others in the Sapotaceae.

Though in foliage and floral structure *Calvaria major* closely resembles several species in the genus Sideroxylon, the fruits and seeds show practically no resemblance to any species in that genus; in fact, the 'stones' or seeds of the two genera are so different that no obvious connecting link appears to exist to show how they may be related.

The genus Calvaria *Commers.* and the species *C. major* Gaertn. f., as has been stated, were founded on the fruit and persistent calyx of a flower, and the only full description of the species is that given in Hooker's 'Icones' (1897). This,

however, is not wholly correct, since Hemsley makes no reference to the tomentum on the young stems, nor to the tomentum on the lower surface of the leaves. Baker (1877) includes *C. major* under *Sideroxylon grandiflorum* A.DC., as a synonym, but from his description it is clear, as Hemsley points out, he has described leaves and flowers of specimens, which though bearing the same name on the collector's label, belonged to a different species from the one described and figured in the 'Icones'. Bouton sent two different plants from Mauritius, both bearing the native name 'Tambalocoque', and he was certainly wrong in thinking *Sideroxylon Boutonianum* A.DC. was the same as *C. major*. These were the specimens which Baker must have seen. De Candolle makes no reference to the fruit of his *Sideroxylon grandiflorum*, but Baker describes it as a 'Drupe the size of a small apple, with a thick fleshy pericarp. Seed depresso-globose 1½ in. thick.' This description, as far as it goes, agrees with the fruit of *C. major*, but not with that of *Sideroxylon Boutonianum*, which from Kew specimens, apparently belonging to this species, are about the size of a large pea and contain an upright seed. *S. Boutonianum* therefore cannot be placed in the genus Calvaria. Since neither Hemsley's nor Baker's descriptions are wholly accurate, an amplified description of *C. major* has been drawn up:

Calvaria major (Gaertn. f.) Hemsley, emend. A. W. Hill. *A tree*, trunk smooth with stout buttresses at the base. *Young stems* densely ferrugineo-tomentose becoming glabrescent with age and grooved, with vertical lenticels; older stems marked by closely packed, horizontal cracks; leaf scars more or less cordate, slightly raised, 3 mm. long and broad. *Leaf petioles* 1·5 to 2 cm. long, finely ferrugineo-tomentose. *Leaves* crowded towards apex of the branches, ovate-oblong, cuneate at the base, apex folded over and rounded, obtuse, emarginate or slightly acute, 9 to 11 cm. long, 4·5 to 6 cm. broad, coriaceous, upper surface shining, lower finely tomentose, especially near and along the midrib, becoming glabrescent with age, nerves and veins prominent on both sides, grooved along the midrib on upper side, leaf margins slightly undulate recurved and thickened. *Flowers* borne on the bare stems, below the terminal cluster of leaves, either singly or in pairs, on slightly raised cushions in the axils of the leaf scars. *Peduncles* 0·6 to 1 cm. long, usually curved and dependent, finely and densely ferrugineo-tomentose. *Sepals* unequal, the two outer thick with crenate margins, 4 to 5 mm. long, pubescent, the inner ones 8 mm. long, 5 mm. broad, concave and rounded at the apex, coriaceous, pubescent on the back, with glabrous and more membranous margins; all sepals pubescent on the inner surfaces. *Corolla* slightly and minutely pubescent on the outside, 5·5 mm. long, tube 1·5 mm., segments rounded, staminodia or coronal filaments 3 mm. long, linear-subulate, margins slightly serrulate, undulate, tips bent over in the bud. *Anthers* epipetalous, 3 mm. long, filaments 3 mm. recurved. *Ovary* densely tomentose, ovoid, 2 to 2·5 mm. long, tapering to the glabrous style, 2 to

2·5 mm. long. *Fruit* 6-locular, spherical, about 5 cm. long by 5·5 cm. broad, somewhat broader above the middle, rounded with a slightly conical apex, somewhat depressed at the base, pericarp smooth and marked by small
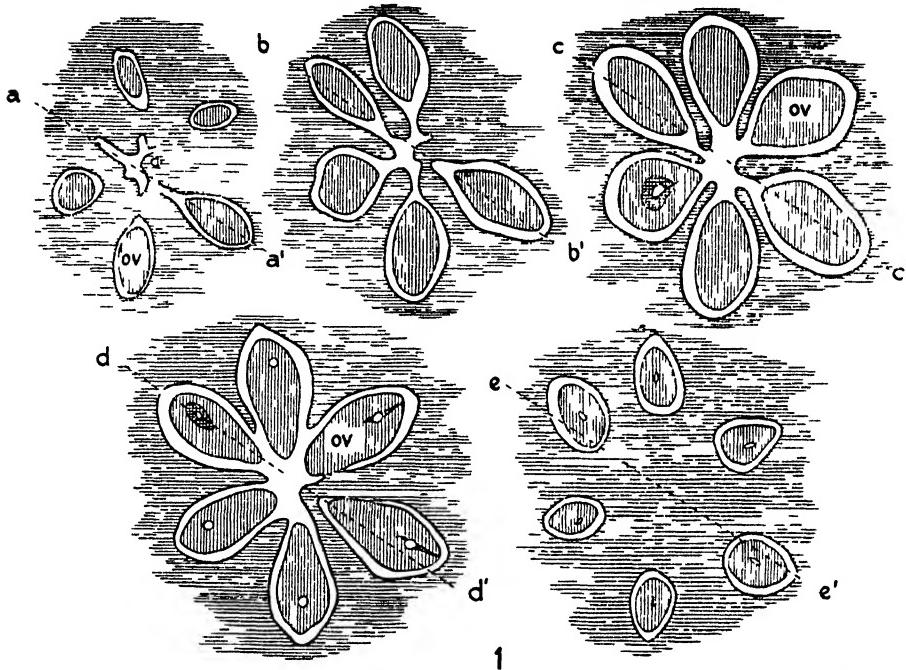
FIG. 1. *Calvaria major.* The ovary in a series of transverse sections. The dotted lines *a—a¹*, &c., refer to the positions of the sections through the ovary—see Fig. 2. *a—a¹* the fused stylar canals in the centre and five ovules (*ov*) one almost connecting with the stylar chamber. *b—b¹* the cavity of four of the ovules connected with the stylar chamber. *c—c¹* all six ovular cavities linked up with the stylar chamber. *d—d¹* one of the ovular cavities now detached. *e—e¹* section near the base of the ovary with the bases of the ovules widely separated by placental tissue.

scattered brown pustules. The persistent and slightly enlarged calyx lies in the shallow depression at the base of the fruit.

The structure of the ovary of *Calvaria major* is of interest and has been studied in serial transverse, and also in longitudinal sections. The style shows six stylar canals, one of which may be V-shaped in transverse section, due to two of them being partly united. Lower down the canals unite into a six-radiate chamber with rounded projections. At about this level the tops of the six loculi with their six ovules can be seen around the periphery of the central area containing the stylar canal cavity. Slightly lower, six projections from this cavity open into the six loculi and all these, which are separated by partition walls reaching nearly to the centre of the ovary, are actually in contact. A little lower down these lateral partitions are shorter and only about half the length of the ovoid ovules, which now lie close together in the central region. In all this upper part of the ovary the six ovules, as seen in transverse section,

are lying perfectly free from the walls of their loculi, and it is at about this stage that the embryo sacs may be seen lying horizontally in the ovules. At a slightly lower level in the series of transverse sections, the attachment of the
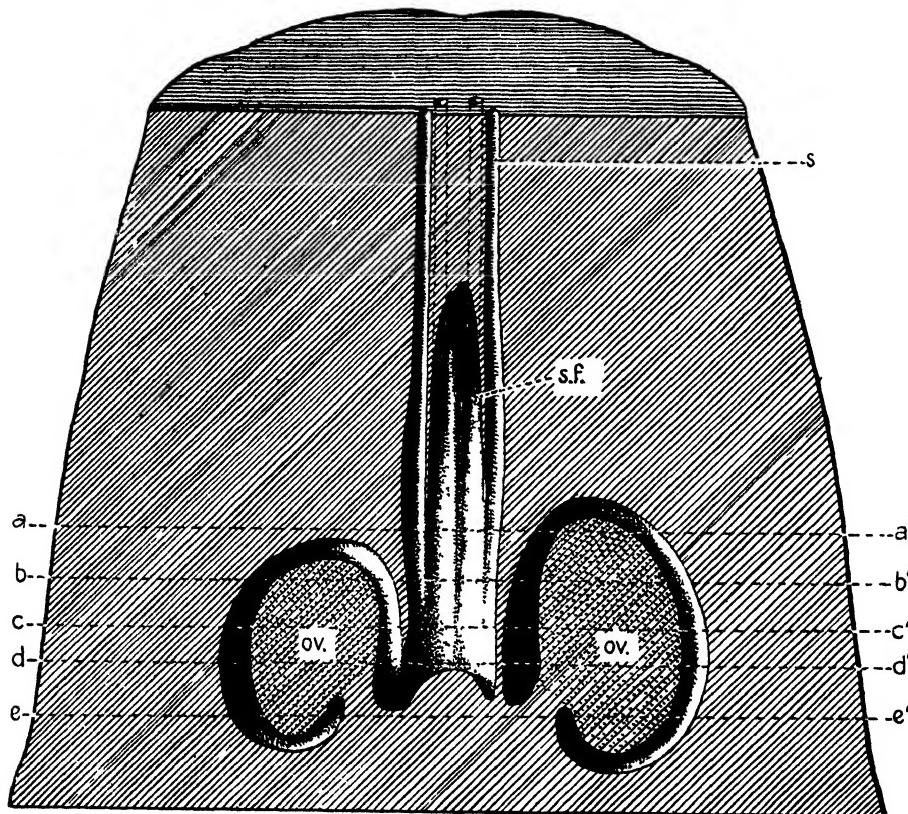
FIG. 2. *Calvaria major*. Diagrammatic view of the style and ovary in longitudinal section to show the independent stylar canals (*s*), which fuse together above the ovules (*s.f.*), and the ovules (*ov.*) in their cavities. The dotted lines *a—a'*, &c., indicate the positions of the transverse sections shown in Fig. 1.

ovules to the partition walls of the loculi, which now meet in the centre, become visible. The ovules are attached laterally by broad funicles to the partition walls close to where they join the floral axis. Sections nearer the base of the ovary show a large central axis, with bundles of vascular tissue and the bases of the ovules lying free in loculi situated at some distance from the centre of the ovary.

The ovary of Calvaria is thus seen to be six-locular in the lower portion, each loculus being a definite cavity separated by a broad partition wall from its neighbour. This arrangement exists to just above the point where the ovules are attached to the partition walls close to the central axis. Above this point the partition walls only reach about half way to the centre, but they meet again where the stylar canals become independent at the top of the ovary.

The six ovules at the time of pollination all appear to be equally developed, but only one of them comes to maturity. The other five disappear and a unilocular cavity results. It seems probable that the surface markings or lobes seen on the polished upper portion of the stony seed may represent mouldings
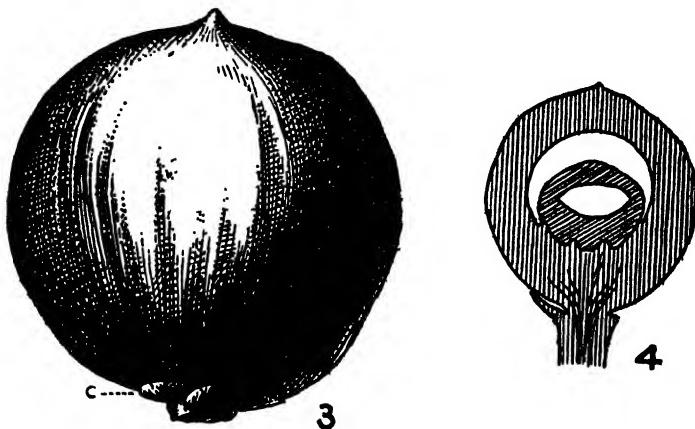
FIGS. 3 and 4. *Calvaria major* Gaertn. f. Fig. 3. A mature fruit with its persistent calyx (*c*) 5 cm. × 5 cm. Fig. 4. An immature fruit in section, showing the stone lying horizontally in the cavity and the attachment of the lower portion to the base of the fruit. (nat. size)

of the upper portion of the loculi into which the one fertile seed extends, the large lobe being a 'cast' of its own loculus and the 5 lobes at the hinder end the impressions made by the other loculi.

Gaertner's figures of the fruit and seed are quite good, but give no hint of the mode of dehiscence on germination, nor does Hemsley in his account in the 'Icones'. This is hardly surprising, as until one can see the hard woody seed actually splitting into two parts at a most unexpected place, no trace of where the fracture may take place can be discerned either by external examination or by sections. Gaertner's figure, which is copied by Hemsley, shows a broadly obpyriform fruit, 6 to 6·5 cm. long, in which the single large stony 'seed' lies horizontally. The fruits recently received from Mauritius, however, have not the pointed apex shown by Gaertner, but are much more rounded and closely resemble a small smooth orange. The large single woody or stony seed lies horizontally in the fleshy mesocarp. Seen from above, the upper surface of the stone is smooth and polished and shows five lobes or undulations at the more rounded end. The micropyle is situated at the slightly more pointed end, but is not visible. The stone or seed measures about 4 cm. long by 3 cm. in breadth.

The upper portion of the stone, as seen from above, is ovoid, rounded and polished and closely resembles the cranium of a human skull. It is larger both in length and breadth than the lower portion or hilum, the surface of which is roughened and deeply pitted.

If the stone be inverted, the smaller lower portion appears like a spherical body lying in a cup—since the upper part projects from 1 to 2 mm. beyond the lower portion, leaving a roughly flat rim all round (Figs. 5, 7, and 9). The whole seed is about 3·5 cm. in thickness, the upper part being 1·5 cm. and the lower 2 cm. thick. The lower portion is furnished further with 8 to 9 irregular pits arranged in the shape of a horseshoe around the conical centre, which is the base of the seed; the open end of this horseshoe of pits being at the micropylar end of the seed as it lies horizontally in the fruit (Fig. 7). The pits contain some fibres, the remains, apparently, of the vascular bundles which connected the seed with the vascular system of the fruit stalk; these bundles are indicated in Gaertner's figure (*b*), where he shows the fruit in section. There is no indication of the place where the seed might be expected to split on germination.

A median transverse section of this double structure along the long axis shows the actual seed lying horizontally in the upper portion of the woody endocarp. The embryo cavity is deeper at the rounded end of the stone, and it is in this depression that the cotyledons lie, the radicle being at the opposite or micropylar end of the cavity, where the stone externally shows a slight point. From the deeper end of the cavity a curved groove can be traced in the woody endocarp leading to one of the pits on the lower side of the stone, and this no doubt marks one of the tracks occupied by the vascular bundles which conveyed food material to the developing embryo. The whole of this lower portion of the seed is a solid woody structure of a fine close texture and takes a beautiful polish when cut in half and planed smooth.

The seed is enclosed in a thin membranous brown testa, which adheres closely to the endocarp and is marked on its inner surface, as seen on the upper side of the cavity, by a fastigiate arrangement of feathery vascular strands curving inwards from the cotyledonary end of the cavity, where they are relatively thick but become thinner towards the micropyle (Fig. 10). On the lower surface of the testa the vascular tissue forms an irregular reticulum spreading from points about the middle of either side (Fig. 11).

These very beautiful and distinct arrangements of the vascular strands in the testa can only be properly seen and appreciated after germination has commenced and the inner surface of the seed cavity can be examined.

In a transverse section of the stone, the endocarp above the seed cavity is 7–8 mm. thick, while below the close-grained, woody stone is from 1·8–2 cm. in thickness, but no sign of the edge of the valve can be seen.

When the seed germinates a flange or valve is thrown off consisting of the greater portion of the upper part of the stone (Fig. 9). The edge of the valve is some 6–7 mm. above the ridge which separates the upper from the lower portion of the stone, but this can only be discovered after germination has commenced, as until then there is no trace of where the split may take place. The split, however, must occur along a definite plane of weakness in the stone, for the valve is a perfectly formed structure and the line of separation is
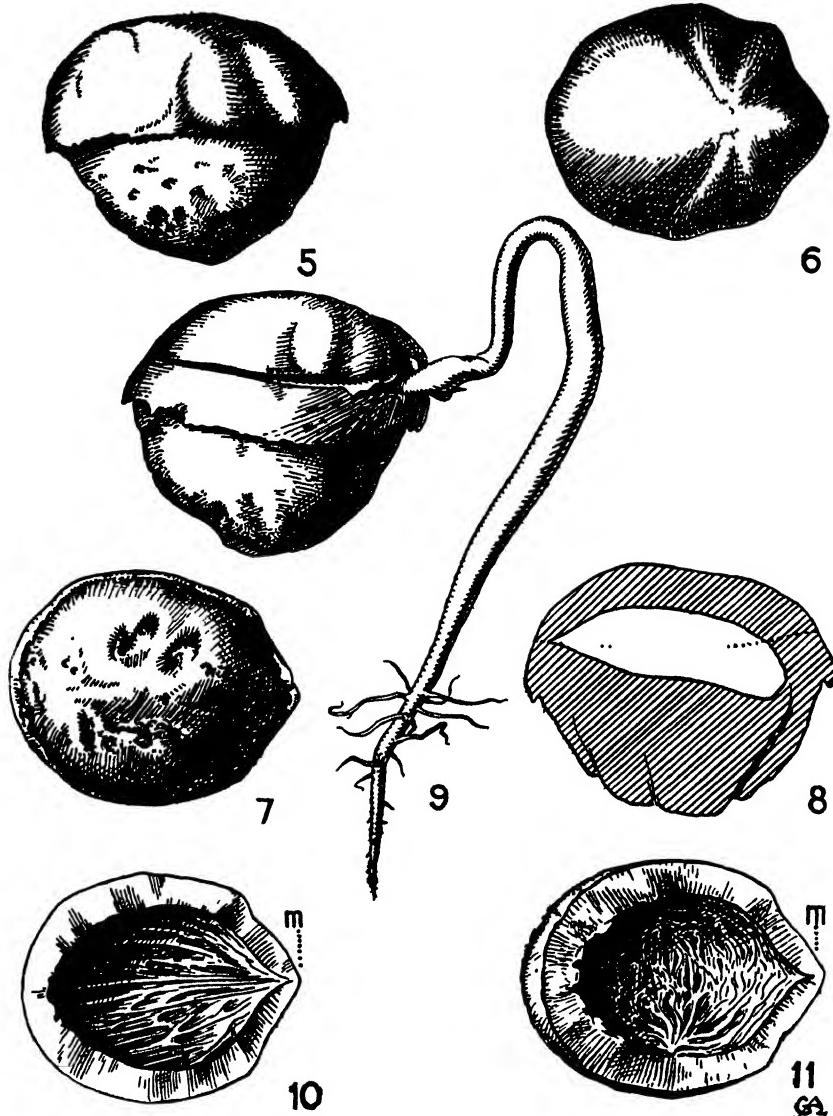
FIGS. 5–11. Fig. 5. The mature stone, in side view, with its polished upper portion showing the lobes and the roughened, pitted, lower portion. Fig. 6. Surface view of the upper portion. The micropylar end of the stone consists of one larger lobe, and there are five smaller lobes at the hinder end. Fig. 7. Surface view of the lower portion to show the stout woody ovoid lower portion lying like an egg on its side in an ovoid cup and with the deep pits arranged like a horseshoe. Fig. 8. The stone in longitudinal section, showing vascular strands in the lower portion and the line of separation of the valve on germination. Fig. 9. A germinating seed showing the splitting off of the valve from the upper portion, some 6–7 mm. above the line of division of the two portions of the stone, also the strongly arched and thickened hypocotyl. In this specimen the lobe at the micropyle end is smaller than usual. Fig. 10. The valve thrown off and viewed from the inside, to show the arrangement of the vascular strands in a fastigiate manner from the micropylar end. Fig. 11. The lower portion of the stone, showing the seed cavity after the valve has been thrown off at germination. The vascular reticulum on this lower surface of the cavity radiates from two points on either side and is at right angles to the arrangement on the upper surface.

definite and regular—the surface exposed by the split being somewhat bevelled or sloping inwards so that the valve fits tightly on to the corresponding surface on the lower portion of the stone, in which about half the seed cavity lies (Figs. 8, 10, and 11). The valve, which is from 5–6 mm. thick, is deeply hollowed, the cavity tapering at the micropylar end into the triangular micropylar orifice. The valve itself viewed on the inside measures 3·7 cm. in length by 3·5 cm. in breadth, the cavity being 3 cm. long by 1·6 cm. broad, and 6–7 mm. deep at the centre. The lower portion of the cavity in the stone is 6–7 mm. deep at the cotyledon tip end, and slopes steeply upwards towards the micropyle (Fig. 8).

The fissure or line of separation between the valve and the lower portion must be laid down during the formation of the stony endocarp, or else the portion split off would certainly show signs of irregular fracture, but the valve is a perfectly formed structure with a firm, definite edge, exactly comparable to the valves thrown off from the 'seeds' of Nyssa and Canarium (Hill, 1933), or of *Panda oleosa* (Hill, 1937), and other similar stony endocarps.

Since the valve is so completely hidden and its margin cannot be detected either externally or in cross-sections of the stone, the force exerted by the swelling embryo on germination must be very great to sever the connexion between the valve and the rest of the stone, even after the moisture from the soil has loosened the substance or ruptured the woody cells which unite the valve to the lower portion of the endocarp.

The stone of Calvaria is composed of small transparent, thick-walled, deeply-pitted cells. Normally, the cells are oblong, but at the spot where the valve is likely to split off they are much more elongated, running in parallel lines both from the micropylar and from the hinder end of the seed cavity towards the periphery, as seen in median longitudinal section. No sign of any plane of weakness between these parallel rows of elongated cells can be seen on microscopical examination of sections of ripe seeds, but the splitting off of the valve must take place by a rupture along the middle lamellae between one or other of these lines of cells.

The smooth polished upper surface of the stony endocarp shows six lobes (Figs. 5 and 6), sometimes clearly, but sometimes only faintly. One of these, at the micropylar end of the stone, sometimes occupies about one-third of the surface and is bounded by slight straight grooves running to the centre of the stone, but in some cases the lobe at the micropylar end is one of the smaller ones. The five other lobes, like the usually larger micropylar one, are convexly curved, triangular in outline with their free sides or margins slightly curved; the grooves separating each lobe run to the centre of the stone where they all meet. These six lobes apparently represent the six loculi of the young ovary, only one of which ultimately develops its ovule (Fig. 4).

On germination the embryo swells considerably and by its pressure ruptures the valve attachment, separating it all along its margin from the lower part of the stone. The radicle then emerges by the micropyle and, when the young

root is anchored firmly in the soil, a very stout and strong curved hypocotyl is developed, forming an arch of from 2–3 cm. in height. The vertical portion of the hypocotyl adjoining the radicle is about 4 mm. in diameter and tapers towards the cotyledons, which are gradually withdrawn by the curvature of the hypocotyl from their oyster-like cavity (Fig. 9).

With regard to the other specimens mentioned at the beginning of this account, the stone of the fruit dug up in Mauritius (p. 589) is much smaller than that of *C. major* and measures about 2·5 cm. in length, 2·1 cm. in breadth, and 1·5 cm. in thickness; the upper surface is flattened, rounded in outline more or less, with almost vertical sides. No distinct lobing can be seen as in *C. major*, but the stone is somewhat pointed at the micropyle. The upper part, which is much larger than the lower, ends with a sharp edge and the lower part, seen from below, is shaped rather like a horse-shoe, with depressions round the edge and measures only 1·3–1·5 cm. in diameter. As the stone is mature it certainly does not belong to *C. major* and from Gaertner's descriptions and figures of *C. hexangularis* and *C. globosa*, it seems to agree with *C. globosa* better than with *C. hexangularis*. Without having been able to see the types, however, the matter must remain uncertain.

No herbarium specimens at Kew can be assigned to either species, with the possible exception of the specimen of a shoot bearing leaves collected by Dr. Philip B. Ayres, received from Mauritius in 1864 (p. 589). This is accompanied on the sheet by half a fruit in a capsule, which measures 1·8 cm. broad by 1·6 cm., with the stony endocarp lying horizontally in the fruit. The leaves are oval-oblong, obtuse, leathery and measure from 14 to 18 cm. long by 5 to 7 cm. broad. The fruit, which it must be presumed belongs to the leaf specimen, since there is a note by it 'seed compressed, cotyledons very thin' in Sir Joseph Hooker's writing, suggests that this specimen may provisionally be referred to *C. globosa*.

The fourth specimen referred to, from the Island of Rodriguez, represents an undescribed species. From the nature of the stony endocarp, it is no doubt a Calvaria, although it differs so markedly in having the stone vertical in the ovoid fruit, instead of lying horizontally as in the other species from Mauritius.

The following description is based on the specimen (No. 1764 in Forest Department Herbarium, Mauritius), a duplicate of which was recently presented to Kew by Dr. R. E. Vaughan, and on a flowering specimen collected by R. Jauffret in Rodriguez, in February, 1941, kindly sent to Kew by Dr. Vaughan. The specific name '*galeata*' refers to the helmet-like appearance of the upper portion of the stone.

Calvaria galeata, *A. W. Hill*, sp. nov. *C. major* Gaertn. f. affinis, sed petiolis longe canaliculatis, foliis oblongo-ovatis juventute utrinque pubescentibus corollae tubo longiore ovario anello piloso instructo fructibus ovoideo-acutis minoribus seminibus erectis praecipue differt.

Tree. **Stems** stout, finely pubescent when young, becoming glabrous and

corky later, with large, pustular, vertical lenticels. Leaves crowded at the ends of the branches. *Petioles* densely pubescent when young, becoming
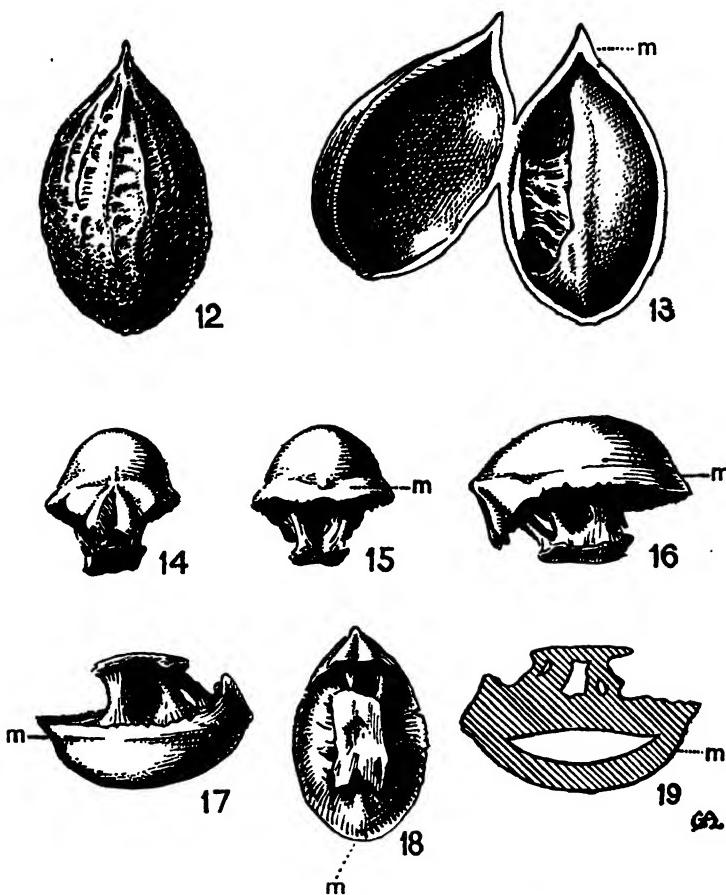
FIGS. 12–19. *Calvaria galeata* A. W. Hill. Fig. 12. The ovoid fruit, covered by the somewhat shrivelled pericarp, 3·3 cm. × 1·9 cm. Fig. 13. The fruit cut open showing the stone lying vertically in the fruit, the small lobes being at the base and reflexed. The micropyle is at the apex of the stone. Fig. 14. The stone showing the four or five lobes from the hinder end. Fig. 15. Front view showing the position of the micropyle (*m*). Fig. 16. Side view to show the lobes turned under below the rim of the stone, and the irregular projections on the under surface. Fig. 17. The stone inverted. The lower woody portion consists of broad strands of woody tissue uniting to form a flat tabular top. Fig. 18. The tabular top on the lower surface seen from above. Fig. 19. The stone in section. The lower portion is very much smaller and more open in structure than in *C. major*.

glabrescent with age, deeply grooved on the upper surface for about 1 cm. adjoining the lamina, 2·5–3 cm. long. *Leaves* ovoid-oblong, 8–13·5 cm. long, 4·5–6 mm. broad, coriaceous, with thickened, slightly recurved, entire margins, rounded or subacute at the apex, rounded or subcuneate at the base, midrib grooved above passing into the grooved petiole, prominent below.

Young leaves densely pubescent on both sides, mature ones becoming glabrous and polished on the upper surface, but hairs retained on the lower surface, veins pinnate, inclined upwards. *Flowers* borne in clusters of 3–5 on cushions in the leaf axils and on the stems; pedicels 1 cm. or more long, slightly
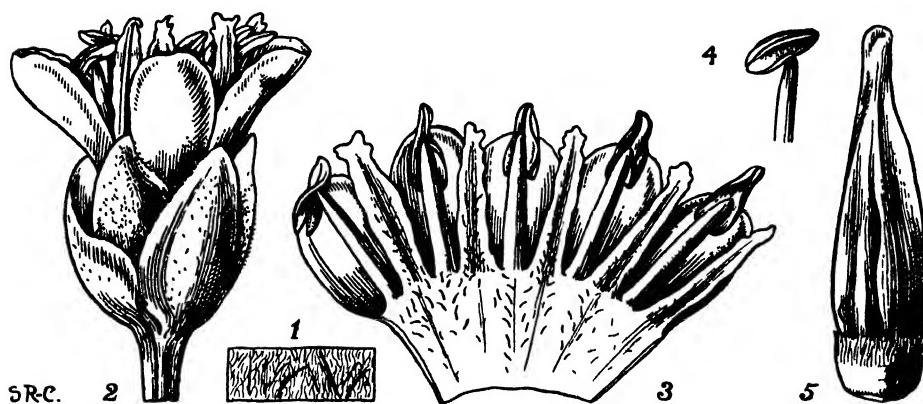
Fig. 20. Flower and portion of leaf of *C. galeata* A. W. Hill; (1) piece of leaf showing the dense tomentum; (2) flower showing the calyx with hairs on the back and margins of the sepals, the rounded corolla lobes, coronal segments and anthers; (3) a flower laid open showing the corona and its pubescence and the epipetalous anthers; (4) an anther; (5) the ovary with the narrow ring of hairs a little above the base.

pubescent, recurved with age and widening below the calyx. *Sepals* 5 mm. long, broadly ovate, subacute, 3·5–4 mm. broad, inner ones minutely hairy and fimbriate on the margins, outer ones less hairy, edges of all sepals membranous. *Corolla* up to 9 mm. long, lobes 5 mm. long, 3 mm. wide, obovate, rounded, tube up to 4 mm. long; corona tube as long as and adnate to the corolla tube, filaments up to 4·5 mm. long, hairy at the base and with scattered hairs in the tube. *Anthers* epipetalous, 2·5 mm. long, dorsifixed, horizontal when ripe. *Ovary* and style conical, 6–6·5 mm. long, ribbed when dry, with a ring of upwardly directed hairs, about 0·5 mm. broad, 0·5 mm. above the base. *Fruit* dependent (?) 3–3.5 cm. long, 1·8–2 cm. broad, ovoid, tapering above to a conical, acute apex, marked in the dry state with numerous longitudinal ridges and grooves. *Pericarp* thin, probably slightly fleshy. *Stony endocarp* lying vertically in the fruit, helmet-shaped, 2·3 cm. long, 1·6–1·7 cm. broad, divided into an upper large smooth helmet-shaped portion and a sharply pointed lower end, where it is marked by 4 or 5 sharp ridges forming the apices of small convex lobes, the whole being smooth and polished with a definite margin forming a rim to the helmet especially at the micropylar end of the stone. The lower portion consists of woody projections rising from the rough floor of the saucer-like lower surface of the upper portion, 5–6 mm. in height, which unite to form a flattened tabular surface measuring 1·1 cm. × 5 mm., more or less rhomboid-cordate in outline,

marked with depressions occupied by vascular tissue arranged somewhat in a horseshoe-like manner.

The lobes at the base of the stone are curved over and lie at right angles to the main helmet-portion of the stone from the point where the ribs unite at its lower end.

RODRIGUEZ. *H. C. King* no. 1764 in Forest Dept. Herb., Mauritius, and in Herb. Kew; Cascade Victoria, *R. Jauffret*, 12.2.41 'Bois de Pomme'; Rodriguez, *Dr. I. B. Balfour* Aug. to Dec. 1874 collected on the Transit of Venus Expedition on the banks of the Rivière Baleine.

C. galeata differs from *C. major* in the longer grooves to the petioles and in the leaves being oblong-ovate rather than obovate, with rounded rather than cuneate bases, and in the smaller ovoid-acute fruits with erect stones.

Dr. Bayley Balfour's specimen, which is referred to by Baker (1877, p. 194) under *C. major*, almost certainly belongs to this new species, for though the leaves are somewhat smaller, they agree in all other respects with King's specimen 1764; the indumentum on all three specimens is of the same type.

The particular point of interest in this Rodriguez species lies in the fruit and seed and especially in the 'seed', which, though agreeing generally in structure with those of the other species of Calvaria, differs in lying in an erect position in the fruit instead of horizontally (Figs. 12 and 13). Then there is the arrangement and position of the lobes or surface markings of the stones. These in the Rodriguez plant are turned under at right angles to the main bulk of the stone and occupy only a very small portion of its surface, almost the whole of the upper portion being unbroken by any division lines, such as may be seen on the upper part of the stone of *C. major* (cf. Fig. 5 with Figs. 14–16). In *C. galeata* on the contrary, the 'lobes' are marked by sharp ridges and individually are much smaller than the corresponding lobes on the stone of *C. major*. The 'under' portion of the stone is also a much less solid, woody structure and is much smaller than in *C. major* (Figs. 13–19).

No seeds of *C. galeata* have been available for germinating, but Dr. Vaughan, writing from Mauritius at the end of June, says, 'In one lot of material I am sending you will notice a seed of the Rodriguez *Calvaria* [*C. galatea*] in which a portion of the woody endocarp has split off just as in *C. major*.' These show the valve splitting off from the helmet portion of the seed and exactly resemble the arrangement shown in *C. major* (Figs. 21, 22).

The fifth species of the genus, the only other one at present known, is *C. imbricarioides* (A.DC.) A. W. Hill from Réunion, to which reference has already been made (see p. 588).

The Sapotaceae have been the subject of monographs and papers by many authors, including Pierre, Baillon, Cordemoy, Engler, Dubard, Baehni, and Lam. The genus Calvaria, founded by Commerson on Gaertner's fruit, is maintained by some and relegated to a section of the genus Sideroxylon by others.

Pierre (1890) appears to regard it as a Sideroxylon, to which genus he gives the name Planchonella. Bentham & Hooker (1873–6) include it in Sideroxylon, merely quoting the Gaertner reference without mentioning the name. Baillon (1890) does not consider Calvaria as a genus distinct from Sideroxylon, but there appears to have been some confusion with regard to specimens from
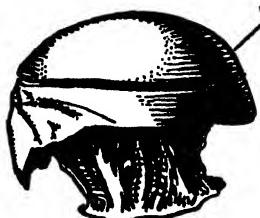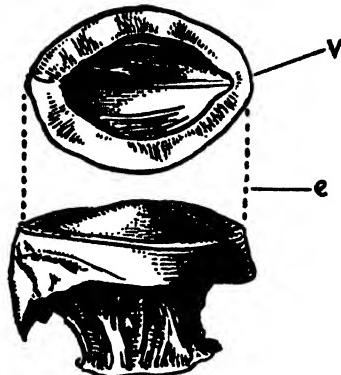
FIG. 21.   A 'seed' of *C. galeata* just received from Rodriguez, showing the line of separation between the valve (*v*), and the lower portion of the stony endocarp.

FIG. 22.   The valve lifted off to expose the under side, showing the embryo (*e*) lying in the cavity in the lower portion of the endocarp.   The valve measures 1·9 cm. long by 1·5 cm. broad, and the wall is 3·5 mm. in thickness.

Madagascar and those from Mauritius. Baillon also seems to suggest that Gaertner's Calvaria is *Sideroxylon Boutonianum* A.DC. In his Histoire (1892) he includes Calvaria under Sideroxylon as a section. Cordemoy (1895) also reduces Calvaria and makes it a section of Sideroxylon. In it he places only the one species found in Réunion, *Sideroxylon imbricarioides* A.DC. Baillon (1892) gives good figures of the 'seed' of Calvaria, one of the lower surface and one in section, which he names *Sideroxylon (Calvaria) globosum*, but they are actually pictures of the seed of *C. major*. Whether they are the seeds to which he refers in his paper in the 'Bull. Soc. Linn. Paris' (1890) or not, he does not say.

Calvaria is maintained as a little-known genus at the end of the family by Durand (1888) with the statement '3 sp. Madagascar ?'. This locality, however, from what we now know, is not correct.

Engler (1890) includes *Sideroxylon imbricarioides* A.DC. in his section Eusideroxylon and, at the end of his account of the family, adds Calvaria *Commers.*, stating that fruits only are known—a one-seeded berry with a horizontally lying seed—and that there are three imperfectly known species in Madagascar. This, while following Durand almost exactly, definitely specifies Madagascar by leaving out the (?). The same particulars are given in the Nachtrag (Engler and Prantl, 1897).

In his monograph of the African Sapotaceae, Engler (1904) follows Corde-

moy in making Calvaria a section of Sideroxylon, but only includes two species, *S. imbricarioides* A.DC. (*S. laurifolium* Commers.), from the Island of Réunion, and *S. grandiflorum* A.DC. (*Calvaria major* Gaertn. f.), described apparently from a specimen collected in Mauritius by A. Daruty, in May 1876, which is preserved in De Candolle's Herbarium. The section Calvaria is separated from his section Eusideroxylon mainly on fruit and seed characters.

Dubard (1912), the next botanist to write on the Sapotaceae, retains Commerson's genus Calvaria and transfers to it eleven species of Sideroxylon—two of which were included in the genus by Gaertner—as well as Hooker's *Cryptogyne Gerardiana* from Madagascar and *Sapota marginata* Dec., from Cape Verde. His genus includes species from the Mascarenes, Madagascar, Mombasa, Socotra, South Africa—(*S. inerme* L.)—Madeira, and Cape Verde! Under *Calvaria Bojeriana* (*Sideroxylon Bojerianum* A.DC.) he includes as a synonym, with a query, *C. globosa* Gaertn., but does not attempt to find a place for Gaertner's other species *C. hexangularis*. He again assigns Commerson's Calvaria to Madagascar, though all our more recent evidence indicates that the specimens come from Mauritius.

Dubard, in defining the genus anew, lays stress on the basal hilum of the seed with the embryo horizontally placed in the seed cavity, the berry being one-seeded. Despite the fact of the importance he attaches to the fruit and seed characters, he admits that unhappily he had not always seeds at his disposal, so has to rely in such cases, on the leaf nervation, and he points out the close resemblance of the flowers to those of Sideroxylon. As fruits and seeds are apparently known in only some five or six of the species he includes under Calvaria, it is clear that far more information is needed before the genus as he defines it can be accepted.

Charles Baehni (1938) is the next to take up the study of the Sapotaceae. He retains Calvaria as a 'genus dubium' with the locality 'Africa'! and at the end of his note says he leaves Calvaria *Gaertn.* in the 'Genera dubia' and reunites Calvaria, *sensu Dubard*, with *Sideroxylon*, citing *C. borbonica* (A.DC.) Dubard as an example. Towards the end of his memoir (l.c., p. 492) he says 'we adopt the idea of Dubard, which consists in restricting the genus only to the species with a basal cicatrice, but we reinstate meanwhile (cependant) *Calvaria*, sensu Dubard'.

Lam, finally, enters the lists, first with a paper on the genus Nesoluma (1938) and secondly with a vigorous criticism (1939) of Baehni's memoir. In the former he sets out a tentative scheme of new sub-divisions of the Sapotaceae in which he makes a new sub-tribe Calvariae of Engler's Sideroxylineae, mainly on the seed structure, and in this sub-tribe he includes the two genera Nesoluma *H. Baill.* and Calvaria *Commers.*, and indulges in interesting speculations as to the probable ancestors of the Calvariae having developed in the Antarctic Continent (l.c., pp. 141-3). In his later paper Lam (1939) under Sideroxylon writes, 'as the type species (*S. inerme* L.) belongs to another group of species ('*Calvaria*') than those which were put to *Sideroxylon* by

Dubard and others, the latter have to receive a new generic name. The species thus far comprised under *Calvaria* have therefore to be named *Sideroxylon*; for the others I would provisionally propose the oldest valid synonym, viz.
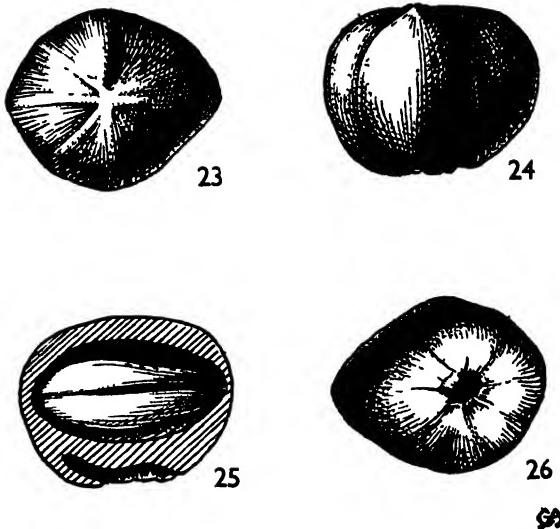
FIGS. 23–6. *Sideroxylon inerme* L. Fig. 23. Stony endocarp viewed from above to show the lobes and ridges. Fig. 24. Endocarp side view, showing three of the lobes. Fig. 25. The same in section with the seed lying horizontally in the cavity and the depressed hilum at the base. Fig. 26. Endocarp, lower surface, to show the oval depressed hilum.

Mastichodendron Jacq. in Hedw. Gen. (1806).' He then lists three certain and two doubtful species which he would place in this new genus. At the end of his paper (l.c., pp. 523, 524) he sets out his suggestion for a natural system as follows:

'Sub-family 1 *Sideroxyloideae* Nov. Subfam. (= tribe *Sideroxylineae* Dub.)
'Tribe A. *Sideroxyleae* Dub. emend. H. J. Lam (= *Calvarieae* H. J. Lam, Bern. P. Bish. Mus. l.c.) seeds subglobose and albuminous, the scar circular or nearly so, basal or sublateral, large or small. Embryo oblique or more or less horizontal. Reticulations of leaves very minutely areolate. Staminodes, if any, usually large or petaloid. (*Nesoluma* Pac. Islands; *Sideroxylon* [= *Calvaria* Comm.] Mascarenes, Madagascar, S. and E. Africa.)'

It has seemed advisable to set out the story of the varying fortunes of Calvaria as a genus, which was founded by Commerson on the remarkable fruits and seeds figured by Gaertner, though for myself, as I am dealing only with the very distinct plants from Mauritius, Rodriguez, and Réunion, I am retaining the genus Calvaria as originally defined, and treating it as a distinct genus based mainly on its remarkable fruit and seed characters, which, as far as can be judged from the often imperfect material, are not found in any species of Sideroxylon proper.

The position of the seed in the fruit, whether horizontal or erect and vertical, raises an interesting point both with regard to *Sideroxylon inerme* and to *Calvaria galeata*, for in *S. inerme* the seed is horizontal (Figs. 23–6), whilst in other species of *Sideroxylon* it is vertical, and in *C. galeata* it is vertical, whereas the horizontal position is characteristic of the genus!

The seeds of *S. inerme* (Plant. Schlecht. 10396, Onrust river, S. Africa, in Herb. Edin.), resemble on a small scale the upper portion of the seeds of Calvaria in their surface lobing and polish, but the lower unpolished part is absent and there is only an oval or rounded, depressed hilum at the base of the seed (Fig. 25). The seeds closely resemble those of other species of Sideroxylon, as regards the hilum, the only difference being that in all the other species, as far as is known, the seeds are obovoid and vertical.

The position of the seed in the fruit, therefore, I do not consider to be a matter of very special importance when other characters are taken into consideration, though Dubard, without having seen seeds of some of the species, lays considerable stress on the point and transfers eleven species to Calvaria, which, except for the two true Calvarias he cites, do not seem to be at all allied to that genus. In the two cases under consideration *C. galeata* from the structure of the seed is obviously a Calvaria though the seed is vertical, whilst *S. inerme* is equally a typical *Sideroxylon*, despite the fact that its seed is horizontal.

I, therefore, propose to reinstate Calvaria as a distinct genus and to include in it *C. major*, *C. hexangularis*, and *C. globosa* from Mauritius, *C. galeata* from Rodriguez, and *C. imbricarioides* from Réunion.

SUMMARY

A description of the stony endocarp of the seed of *Calvaria major* from Mauritius is given and of its mode of germination; a portion of the upper skull-like part of the thick endocarp being thrown off. An account is also given of the somewhat similar fruit and seed of a new species of Calvaria from Rodriguez which has the seed vertical in the fruit instead of horizontal. This new species, *C. galeata*, is described, making five species in the genus, which are distributed as follows:—*C. major*, *C. hexangularis* and *C. globosa* in Mauritius, *C. galeata* in Rodriguez, and *C. imbricarioides* in Réunion.

The status of the genus is discussed and a review given of the opinions of the various authorities as to its relation to Sideroxylon, and the conclusion is reached that Calvaria should be maintained as a genus allied to, but distinct from, Sideroxylon, mainly on account of its remarkable stony endocarp.

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Studies in Tropical Fruits

XIII. Carbohydrate Metabolism of the Banana Fruit during Storage at 53° F. and Ripening at 68° F.

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With twelve Figures in the Text

I. Introduction

THIS work is a sequel to previous studies (Barnell, 1940, 1941) on some aspects of the biochemistry during development of the Gros Michel banana fruit and during its ripening under tropical conditions. The subject for the present investigation has been the changes which occur in the carbohydrates of fruit cut at '¾-full'[1] and 'heavy ¾-full'[1] grades and given periods of cold storage approximating to those required for transportation to England and Canada respectively, followed by standard ripening procedure. The effect of a prolongation of the cold storage for each grade has also been investigated so that some aspects of the chemical nature of the abnormalities in the eating and other qualities associated with 'chilled' fruit could be observed.[2]

Previous work, necessarily mainly confined to fruit in the later stages of its metabolism, is noteworthy for the conflicting nature of the observations presented. In many cases the variety examined was not named, e.g. Buignet (1861), Corenwinder (1876), Ricciardi (1882), Gerber (1896), Prinsen-Geerligs (1908), Yoshimura (1911), Bailey (1912), Thompson and Whittier (1912), Gore (1914), Myers and Rose (1917); though in the later investigations it may, perhaps, be presumed that Gros Michel was selected. Fruit was obtained from localities (usually unspecified) of very different soil and climatic conditions, and examined after receiving varying conditions and periods of transport, refrigerated or otherwise. The grade of fruit almost invariably has not been given. The methods of treatment and of analysis have been as various as the material; the neglect of speedy de-activation of the enzyme system of the fruit, as in those investigations in which cold water or warm alcohol were used for extraction of sugars, renders some of the data obtained in such investigations open to considerable suspicion, e.g. Reich (1911), Thompson and Whittier (1912), Gore (1914), Stratton and Loesecke (1930). In those investigations in which apparently satisfactory extraction methods have been used there is little similarity between the values obtained by various workers for concentrations of total sugars and of sucrose; this must partly be accounted for by the variety of analytical methods used but is also probably due, to a large extent, to the variable material. That there may be a considerable variation in the composition of fruit of a single variety grown in different soils and under different climatic conditions, has been shown for apples by Archbold (1928) and Kidd, Onslow, and West (1929).

It is clearly necessary that biochemical studies of banana ripening should contain reference to the variety, origin, grade, and treatment of the fruit if comparisons are to be made with further work. Isolated sets of analyses of the

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[1] For explanation of these terms used by the banana trade see Wardlaw, Leonard, and Barnell, 1939 *a*.

[2] The general characteristics of chilling and the storage conditions which produce it are described in one of the earliest publications from this station (Wardlaw and McGuire, 1931). More recently an account has been given of the respiration of detached fingers during storage at 53° F. and subsequent ripening at controlled temperatures (Leonard and Wardlaw, 1941).

carbohydrate composition of 'ripe' bananas are of little value, as a wide range of starch, sucrose, and reducing sugar values occurs within the pulps of fruits popularly defined as 'ripe'.

II. MATERIALS AND METHODS

Bunches of 'standard ¾-full' and 'heavy ¾-full' fruit were obtained from the Toco district of Trinidad. The initial values for mean finger weight and pulp/skin ratio were: standard ¾-full, 144·0 gm. and 1·48; heavy ¾-full, 163·1 gm. and 1·60; cf. Wardlaw, Leonard, and Barnell (1939 *a*). Each bunch was wrapped in banana trash to reduce damage during transit from the estate and was delivered at the Research Station within six or seven hours of cutting.

Thirty closely uniform bunches of each grade were selected for each treatment (short and long storage period), i.e. 120 bunches in all. One sample was taken on the arrival of the fruit at the laboratory and two more within the first 24 hours, after which samples were taken after suitable intervals.

The method of sampling and of the preparation of samples for analysis remained as described in a previous paper in this series (Barnell, 1941); the methods employed for the extraction and estimation of carbohydrates have also previously been recorded (Barnell, 1936, 1940).

A complication due to sampling was introduced into the records of the long storage fruit of both grades which appears to a greater or less extent in all series of data for such fruit. After the ¾-full fruit had been sampled for 17 days and the heavy ¾-full fruit for 14 days sampling was changed from upper-row fingers of the third hand of each bunch to lower-row fingers of the same hand. These fingers were smaller and more curved and their lower weights produce a discontinuity at the time of the sampling change in the drifts of data based on the weights of fingers.[1]

III. BEHAVIOUR OF FRUIT DURING STORAGE AND RIPENING

Sixty bunches of ¾-full fruit were placed in a storage room approximately 26 hours after arrival. They were cooled to an air temperature of 53° F. within 24 hours; 30 bunches were maintained at this temperature for 14 days (short storage) and the remaining bunches for 20 days (long storage). At the end of each respective period of cold storage the bunches were removed to a ripening room at 68° F.

Of the 30 bunches receiving the short storage treatment none showed signs of ripening before removal to the ripening room; after 2 days at 68° F., 2 out of the 30 bunches had fingers softening while after 4 days 29 bunches had yellowing fruit; by 6 days all bunches were yellow or yellowing (some green hands remaining on 10 bunches); 5 days later (11th day in ripening room) the fingers of all bunches were showing anthracnose spotting and by the

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[1] If the changes had been made to upper-row fingers of an adjacent hand less variation would have been experienced (Wardlaw, Leonard, and Barnell, 1939 *a*).

13th day of ripening the spots were coalescing and the fingers falling from the bunches.

Though some stem-end rot was present it was not sufficient to cause wastage. No signs of chilling were observed in any of the fruit.

Two of the bunches receiving the long storage treatment began to ripen (softening and colouring) within 13 days of cold storage and were removed as suspected Cercospora-infected fruit (Wardlaw, 1937). Two more showed signs of ripening by the 16th day but were not removed, a further 2 had 'sprung' by the 20th day, on which day the 28 remaining bunches were removed to the ripening room. After 2 days at 68° F. 19 out of the 28 bunches were yellowing and by the 4th day all 28 were yellow, 26 showing chilling discoloration. By the 9th day anthracnose spots had developed on all and chilling symptoms, i.e. a dull yellow followed by a russet colour of the skin,[1] were now observed in the remaining 2 bunches. Fingers were falling from the bunch by the 12th day. The stem-end rot present was not sufficient to cause wastage.

The 60 bunches of the heavier grade of fruit (heavy ¾-full) were placed in cold store approximately 23 hours after arrival and were cooled to an air temperature of 53° F. within 22 hours. The short storage bunches were removed to the ripening room at 68° F.[2] after 7 days and the remaining 30 after 14 days in store. Of the bunches subjected to the short storage treatment 1 began to show signs of ripening after 6 days, this was removed; by the 8th day another bunch had 'sprung'. After 3 days at 68° F. 9 bunches were yellowing, after 6 days there were 26 ripe or nearly ripe bunches, while by the 10th day all were yellow with anthracnose spotting on the majority. Of the long storage bunches only 1 was showing indication of ripening by the 15th day when the fruit was transferred to the ripening room. After 2 days at 68° F. 10 bunches were ripening, and after 6 days all 30 bunches were ripe, most of them showing chilling symptoms (23 bunches) and many (19) having anthracnose spotting. By the 10th day the fingers were blackened with coalescing anthracnose spotting and were falling. Stem-end rot, though present, caused no wastage.

IV. The Pulp/Skin Ratio

During ripening, the pulp increases in fresh weight, obtaining water from the skin (Gore, 1914; Smith, 1932; Barnell, 1941) and possibly the stem (Stratton and Loesecke, 1931) by virtue of the high suction pressure created by the high concentrations of osmotically active substances, particularly sugars. The skin loses weight by loss of water to the pulp and also to the

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[1] Fuller details of chilling symptoms of the Gros Michel banana may be found in the paper by Wardlaw and McGuire, 1931.

[2] A ripening room temperature of 65° F. is more usual for this grade, but in the present instance a temperature of 68° F. was used in order that direct comparisons could be made with the ¾-full fruit.

atmosphere, the latter rate of loss depending on the humidity and temperature. This differential behaviour of the pulp and skin during ripening causes a progressive drift in the ratio of pulp to skin weight with the degree of ripeness of the fruit. This relation, under the title of 'coefficient of ripeness', was first introduced by Tallarico (1908) as an index of fruit maturity: it has since been employed by several authors—Bailey (1912), Bourdouil (1929), Wardlaw, Leonard, and Barnell (1939 *a*)—and has been criticized, on scanty evidence, by Wolfe (1931) as 'unreliable as well as insensitive'.

TABLE I

Variation in the Pulp/Skin Ratio during Storage and Ripening

⅔-full fruit.					Heavy ⅓-full fruit.				
Days from cutting.	Temp. (° F.).	Mean pulp wt. (gm.).	Mean skin wt. (gm.).	Pulp/ Skin.	Days from cutting.	Temp. (° F.).	Mean pulp wt. (gm.).	Mean skin wt. (gm.).	Pulp/ Skin.
	Room temp. (80–5)					Room temp. (80–5)			
0	(80–5)	85·8	58·2	1·48	0	(80–5)	100·3	62·8	1·60
20 hr.	,,	84·9	57·4	1·48	16 hr.	,,	100·5	62·3	1·62
26 hr.	53	84·7	57·3	1·48	23 hr.	53	99·5	62·0	1·61
3	,,	85·2	56·3	1·51	2	,,	98·4	60·2	1·64
6	,,	83·2	56·5	1·48	4	,,	98·7	60·5	1·63
10	,,	84·2	56·9	1·48	6	,,	96·4	60·2	1·60
14	,,	84·6	56·7	1·49	9	,,	96·5	59·0	1·64
17	,,	76·3	48·2	*1·58	11	,,	97·2	59·0	1·65
19	,,	74·7	45·8	1·63	14	,,	84·6	48·0	*1·77
21	68	76·2	46·3	1·65	16	68	84·8	46·7	1·82
23	,,	78·9	46·7	1·69	17	,,	94·7	56·1	1·69
25	,,	82·2	43·6	1·89	19	,,	91·7	52·4	1·75
27	,,	82·0	37·4	2·20	21	,,	88·9	43·2	2·06
29	,,	87·5	36·8	2·38	24	,,	95·2	38·8	2·46
31	,,	87·4	34·9	2·50	26	,,	98·5	35·3	2·79
33	,,	90·3	33·8	2·67	—	—	—	—	—
16	68	85·0	56·2	1·51	9	68	94·8	58·3	1·63
18	,,	85·7	56·1	1·53	11	,,	94·1	58·8	1·60
20	,,	85·8	50·8	1·69	13	,,	95·3	56·2	1·70
22	,,	88·3	47·4	1·87	16	,,	97·7	49·7	1·97
24	,,	89·2	43·7	2·04	18	,,	99·2	45·9	2·16
26	,,	90·6	39·8	2·28	—	—	—	—	—
28	,,	92·7	38·3	2·42	—	—	—	—	—

The lower section of the table shows the data obtained during the ripening of fruit removed from low temperature storage before any 'chilling' had occurred. The mean weights of pulp and skin were derived generally from 30 observations but in later instances from 29 and 28 when suspected Cercospora-infected bunches had been removed.

* The asterisk before a pulp/skin ratio indicates that that value and those following it in the same column were obtained from fingers of the lower rows of the 3rd hands of the bunches sampled; preceding values were obtained from upper-row fingers.

Table I sets out values for mean pulp weights and mean skin weights for ⅔-full and heavy ⅓-full fruit during long and short cold storage periods

followed by ripening at 68° F. with approximately 85 per cent. relative humidity. The ratios of pulp and skin weights are also given in the table; these values are seen to increase slowly throughout the cold storage period, the initial value for ¾-full freshly cut fruit being 1·48 and for heavy ¾-full 1·60. The rate of increase was somewhat accelerated when the fruit was placed in the ripening rooms, but a relatively sudden rise from values of 1·80–1·90 to values of above 2·00 occurred at the stage when the fruit became 'ripe'. The approximate coincidence of this index of ripeness with the stage assessed as eating-ripe by observation and chemical analysis will be shown later (section XI).

A point of difference arises between chilled fruit (i.e. fruit which had received too long a period of cold storage for subsequent satisfactory ripening) and unchilled fruit. The gain in weight of the pulp during the ripening period at 68° F. was 14·1 gm. and 13·7 gm. for chilled ¾-full and heavy ¾-full fruit respectively, while for unchilled fruit the weight increases were 7·7 gm. and 4·4 gm. respectively. The corresponding losses in weight of the skin were 12·5 gm., 11·4 gm. for chilled fruit and 17·9 gm., 12·4 gm. for unchilled fruit. So either considerable passage of water from the stem to the pulp or a reduced transpiration rate or both occur in chilled fruit. The pulp retains more water in the chilled fruit during ripening than in the unchilled fruit for approximately similar total losses by the skin, so the general level of pulp/skin ratio values for chilled fruit during ripening is higher than for unchilled (see Table I). The corresponding losses of total dry matter from the pulp were such as to present final values of pulp water content of the over-ripe fruit, chilled and unchilled, of approximately equal magnitudes: ¾-full, chilled, 80·60 per cent., unchilled, 80·52 per cent.; heavy ¾-full, chilled, 79·96 per cent., unchilled, 79·62 per cent.

TABLE II

Weight-Changes in Pulp, Skin, and whole Fingers
Total change over whole Ripening Period

	¾-full fruit.			Heavy ¾-full fruit.		
Condition of fruit	Whole finger. (gm.).	Pulp. (gm.).	Skin. (gm.).	Whole finger. (gm.).	Pulp. (gm.).	Skin. (gm.).
Unchilled	141·2	85·0	56·2	153·1	94·8	58·3
	131·0	92·7	38·3	145·1	99·2	45·9
	−10·2	+7·7	−17·9	−8·0	+4·4	−12·4
Chilled	122·5	76·2	46·3	131·5	84·8	46·7
	124·1	90·3	33·8	133·8	98·5	35·3
	+1·6	+14·1	−12·5	+2·3	+13·7	−11·4

This effect of chilling on the water relations is shown in Table II where total weight-changes over the ripening period are given for the whole finger,

the pulp and the skin of both grades of fruit, chilled and unchilled. The data are not susceptible to statistical analysis, so must be regarded as merely indicating the noted effects. As a generalization from the data of Table I it may be said that in unchilled fruits less than half of the total weight loss of the skin is accounted for by the increase in pulp weight, the remainder being loss in weight by the whole finger (by transpiration). In chilled fruit all the loss in weight of the skin is accounted for by the increase in pulp weight and the data indicate no appreciable loss in weight of the entire finger (actually a small gain was observed); water lost by the finger was, presumably, mainly drawn from the stem and probably transpiration was much lower than in unchilled fruit.

V. PERCENTAGE AMOUNTS OF DRY MATTER AND VARIOUS CARBOHYDRATES IN THE PULP

The percentage amounts of dry matter and of the estimated carbohydrates in the pulp are graphed for ¾-full fruit in Figs. 1 and 3 and for heavy ¾-full fruit in Figs. 2 and 4. In each figure values for both long storage (chilled) and short storage (unchilled) fruit are plotted.

(a) *Total dry matter, starch, and total sugars.*

Apart from the first three observations, the percentage of dry matter in the pulp of ¾-full fruit (Fig. 1) fell slowly and steadily throughout the period of cold storage. The rise on placing in cold storage was, partly at least, due to the high transpiration losses of the cut bunches during the initial period at tropical temperature. After 21 days from the receipt of the fruit, i.e. after 20 days in cold storage, removal to the ripening room resulted in a much accelerated decline in the percentage dry matter content due to absorption of water from the skin as the suction pressure increased with increasing sugar concentration and also to the accelerated rate of loss of dry matter in respiration at this stage. After 15 days (14 days' cold storage) the short storage fruit was transferred to the ripening room and the subsequent drift of its percentage dry matter content is shown by the broken curve. Starting at a slightly higher level than the long-stored fruit, though only just significantly different from it at the corresponding stage of storage, the drift during the ripening phase was in all respects similar to and the final percentage attained in over-ripe fruit almost the same as that for the long-stored fruit; viz. 19·48 and 19·40 respectively.

The starch percentage, shown in the same figure, fell during the interval preceding entry into cold store but during the first 9 days of storage showed a not inconsiderable, significant increase suggesting possible resynthesis from some temporary unestimated reserve. From the 10th day declining values ensued, the rate of decrease becoming accelerated after the 17th day, though the fruit was still at 53° F. On transferring to the ripening room the
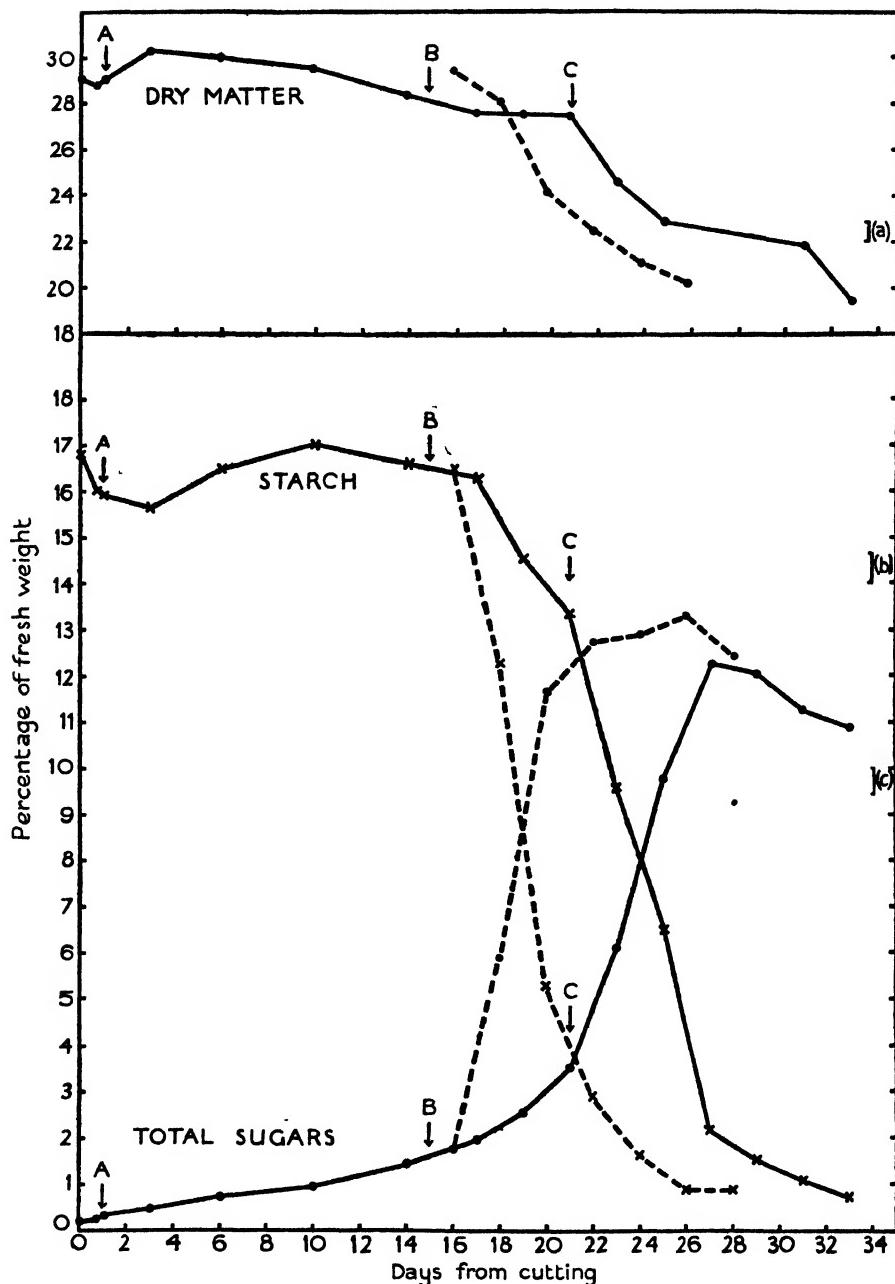
FIG. 1. Percentage amounts of dry matter, starch, and total sugars in the pulps of '¾-full' bananas during storage at 53° F. and ripening at 68° F. The vertical arrows labelled A indicate the time of placing the fruit in store; those labelled B and C the end of the storage period for short and for long storage fruits. The arrows retain this significance in all subsequent figures. The ripening changes of the short storage fruit are shown by discontinuous lines in this and

familiar rapid starch hydrolysis curve of ripening banana pulp occurred. In the fruit subjected to the shorter storage term the accelerated starch degradation in cold store had not begun at the time of transference (15 days) to the ripening room. The subsequent rate of fall of the starch percentage during ripening was somewhat greater with this fruit than with the long-stored fruit, suggesting that 'chilling' partially deranges the starch hydrolysis mechanism of the banana pulp (see also section XII and Table VII). The final starch percentage attained was, however, about the same in both instances.

Total sugars increased in percentage amount steadily throughout the storage period. When the short storage fruit was transferred from cold storage to the ripening room on the 15th day the rate of increase of total sugars became very rapid (broken line of total sugars in Fig. 1); the sugars in the long storage fruit also increased more rapidly after 16 days until by the 21st day, when transference to the ripening room occurred, the curve (continuous total sugars curve in Fig. 1) was well on the rapid upward trend and continued, with no abrupt change, during the ripening phase. This beginning of the accelerated rise of total sugar content between the 16th and 21st days together with the coincident starch degradation while the fruit was still in cold storage at 53° F. shows that the fruit can enter relatively suddenly upon its fast-ripening phase during storage at this temperature. Respiration data at 53° F. show a similar relatively rapid initiation of the climacteric (Leonard and Wardlaw, 1941). It is probable that it is in this partially ripened state that the fruit is subject to 'chilling' and the consequent abnormalities of that state. The total sugar content reached a higher maximum in the ripe unchilled fruit than in the chilled 'ripe' fruit by 1 to 2 per cent.

Chilling did not occur in the fruit removed from cold store on the 16th day, and from examination of the starch and total sugar curves of Fig. 1 it would appear that 16 to 18 days represent the maximum time of storage (15 to 17 days of actual cold storage) which can be given $\frac{3}{4}$-full fruit without risk of chilling.

Fig. 2 provides similar data to those given above, for heavy $\frac{3}{4}$-full fruit, the grade normally exported from Trinidad to Canada. The fruit in this instance received nearly a full day at tropical temperatures from the time of cutting before entering cold store: the short storage fruit was removed after 7 days' cold storage (8th day) and the long storage fruit after 14 days (15th day). The dry matter curve for the long storage fruit (continuous line in upper portion of figure) showed fluctuating values up to the 14th day and then, after the fruit was placed in the ripening room, fell steadily throughout the ripening

all the figures. The vertical lines labelled (*a*), (*b*), and (*c*) represent the minimum significant differences ($P = 0.05$) of (*a*) dry matter, (*b*) starch, and (*c*) total sugars.

The short storage (14-day) fruit remained green till day 15, yellowing occurred till day 19, after which the fruit was 'eating-ripe' till day 2 and then became over-ripe. The corresponding stages for the long storage (20-day) fruit were: green till day 20, yellowing to day 24, ripe from day 24 to day 29, afterwards over-ripe.
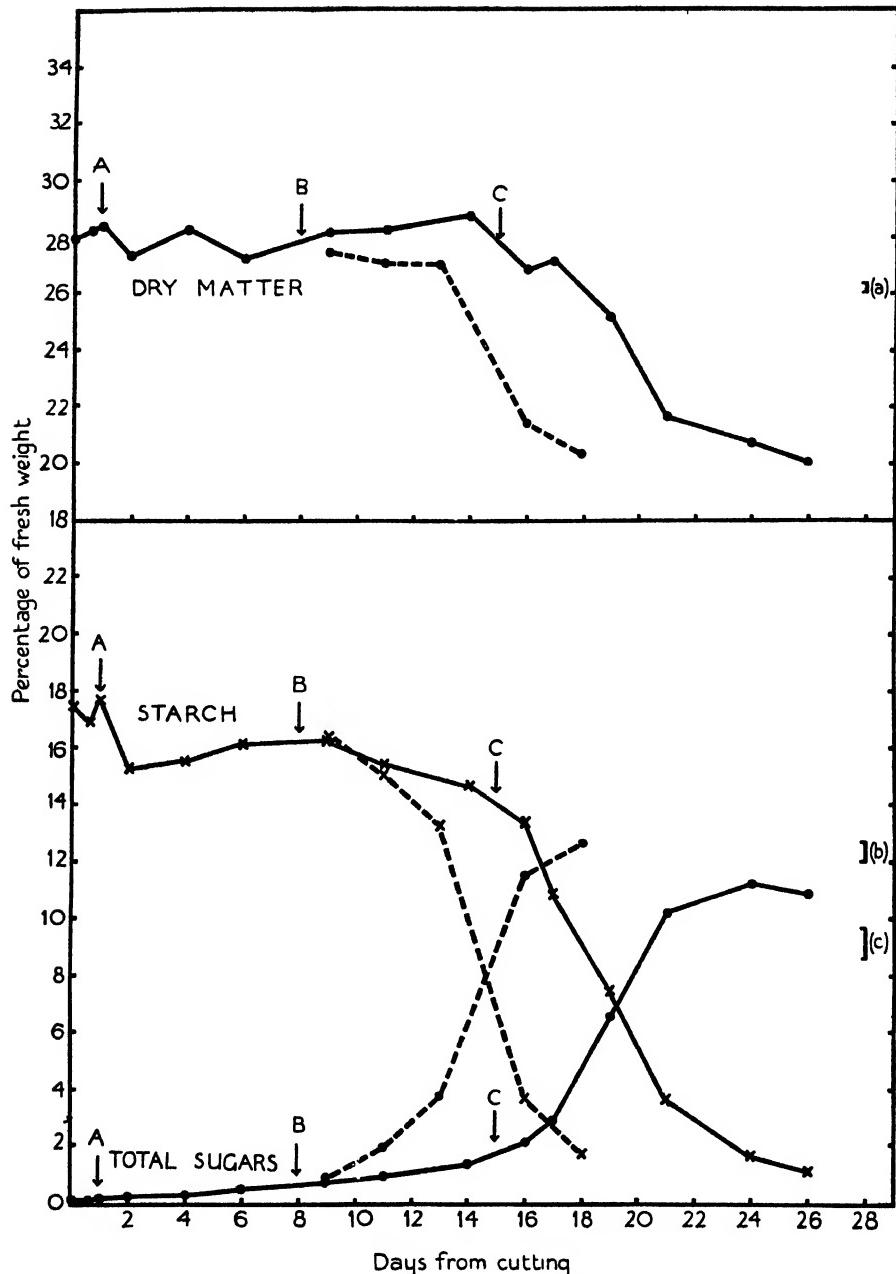
FIG. 2. Percentage amounts of dry matter, starch, and total sugars in the pulps of 'heavy ¾-full' bananas during storage at 53° F. and ripening at 68° F. The vertical lines labelled (a), (b), and (c) represent the minimum significant differences (P = 0·05) of (a) dry matter, (b) starch, and (c) total sugars.

The short storage (7-day) fruit remained green till day 10, yellowing occurred till day 14 after which the fruit was 'eating-ripe' till day 18 and then became over-ripe. The corresponding stages for the long storage (14-day) fruit were: green till day 14, yellowing to day 19, ripe from day 19 to day 23, afterwards over-ripe.

period: the short storage fruit after transference to the ripening room showed little change in the dry matter content of the pulp for 5 days (13th day from cutting of fruit) and then fell rapidly as ripening proceeded. As in the ¾-full fruit the final percentage attained was approximately the same in long- and short-stored fruit.

The total sugar and starch percentage contents of the pulps behaved as described for the ¾-full fruit, allowing for time differences due to the use of heavier grade and therefore more early-maturing fruit. The starch content again fell slightly more rapidly at 68° F. in the short storage than in the long storage (chilled) fruit but reached approximately the same level (see also section XII and Table VII): the maximum percentage of total sugars attained in the ripe pulp was again 1 to 2 per cent. higher in the unchilled fruit than in the chilled.

(b) Sucrose, glucose, fructose, and glycosidic-glucose.

The percentage contents of these carbohydrates in the pulp are given in Fig. 3 for ¾-full fruit and in Fig. 4 for heavy ¾-full fruit.

In both grades sucrose began to increase during the first day while the fruit was at tropical temperatures awaiting storage. During the period of cold storage a steady rate of increase occurred until the removal of the short storage fruit to the ripening room; the sucrose percentage in this fruit then quickly increased to nearly 4 per cent., the rise being quicker in the ¾-full than the heavy ¾-full fruit. The maximal sucrose concentration occurred in each instance during the eating-ripe stage. The long storage fruit showed accelerated sucrose increase rate 3 days after the removal of the short storage fruit (i.e. 17 days from cutting in the ¾-full and 11 days from cutting in the heavy ¾-full) until its own removal, again indicating that the phase of acceler-ated ripening had begun during the prolonged cold storage. On removal to the ripening room the sucrose increase continued with only a slight increase in rate (Figs. 3 and 4). In both the ¾-full fruit (Fig. 3) and the heavy ¾-full fruit (Fig. 4) the maximum sucrose concentration attained in the normal unchilled (short storage) fruit was slightly higher than in the chilled (long storage) ¾-full fruit and considerably higher in the heavier grade of fruit in which the chilling was more severe than in the thinner grade.

Glucose and fructose were present in very small, approximately equal amounts in the fruit as received, and continued so during the periods of cold storage, apparently mainly as invert sugar derived from sucrose. A slow increase in the percentage amounts of both sugars took place during the cold storage period (Figs. 3 and 4) with an accelerated rate of increase during the last 4 days in the long storage fruit (17th to 21st day in ¾-full, 11th to 15th day in heavy ¾-full). When the short storage fruit was transferred to the ripening room a rapid increase in the reducing sugar percentages took place with glucose increasing to higher values than fructose in the ¾-full fruit, Fig. 3, though this latter effect was not observed in the heavy ¾-full fruit, Fig. 4.

The long storage fruit showed similar rises of glucose and fructose following transference to the ripening room but did not attain such high maximal values in the short storage fruit of the ¾-full grade (Figs. 3 and 4). Final
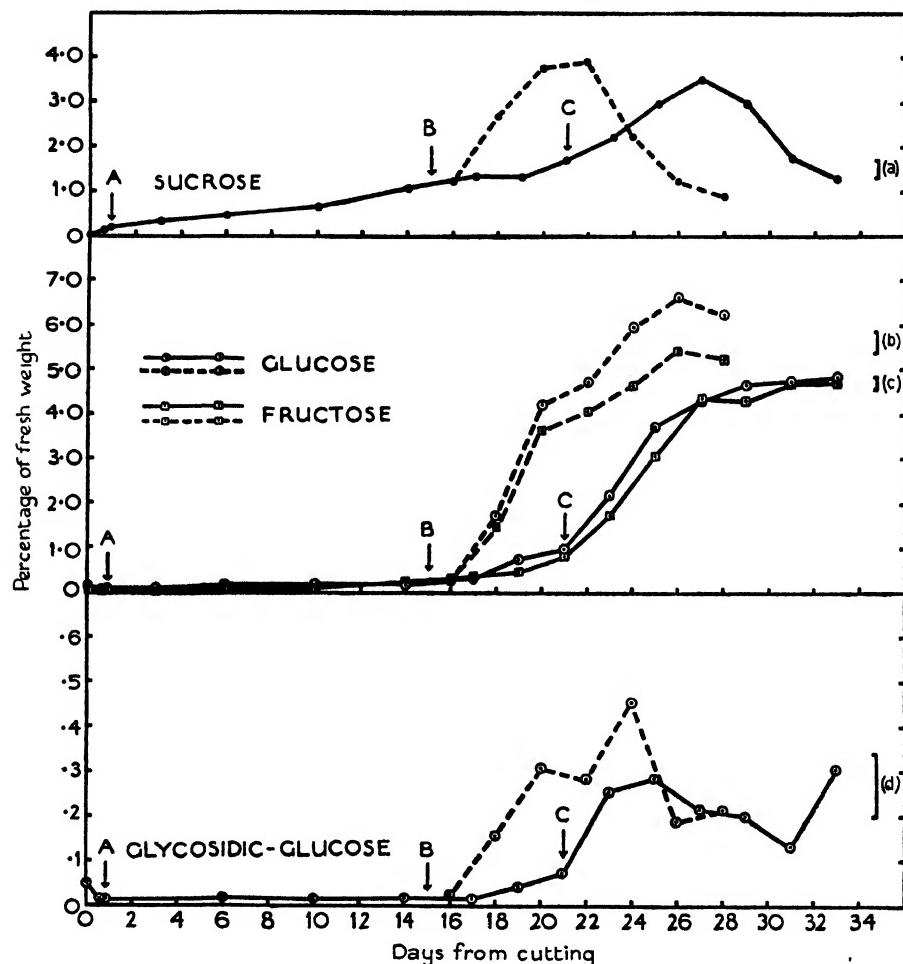
FIG. 3. Percentage amounts of sucrose, glucose, fructose, and of glycosidic-glucose in the pulps of '¾-full' bananas during storage at 53° F. and ripening at 68° F. The vertical lines labelled (a), (b), (c), and (d) represent the minimum significant differences (P = 0·05) of (a) sucrose, (b) glucose, (c) fructose, and (d) glycosidic-glucose.

For a description of the ripening record of '¾-full' fruit see the subscript to Fig. 1.

values for fructose were, on the whole, lower than those for glucose, particularly in the heavy ¾-full fruit (Fig. 4).

During the later stages of maturity when visible ripening was taking place there was evidence of glycoside synthesis. In Fig. 3 (bottom portion) the drift of 'bound' or glycosidic-glucose is given; a considerable increase in its percentage amount was observed on transferring the short storage fruit to the
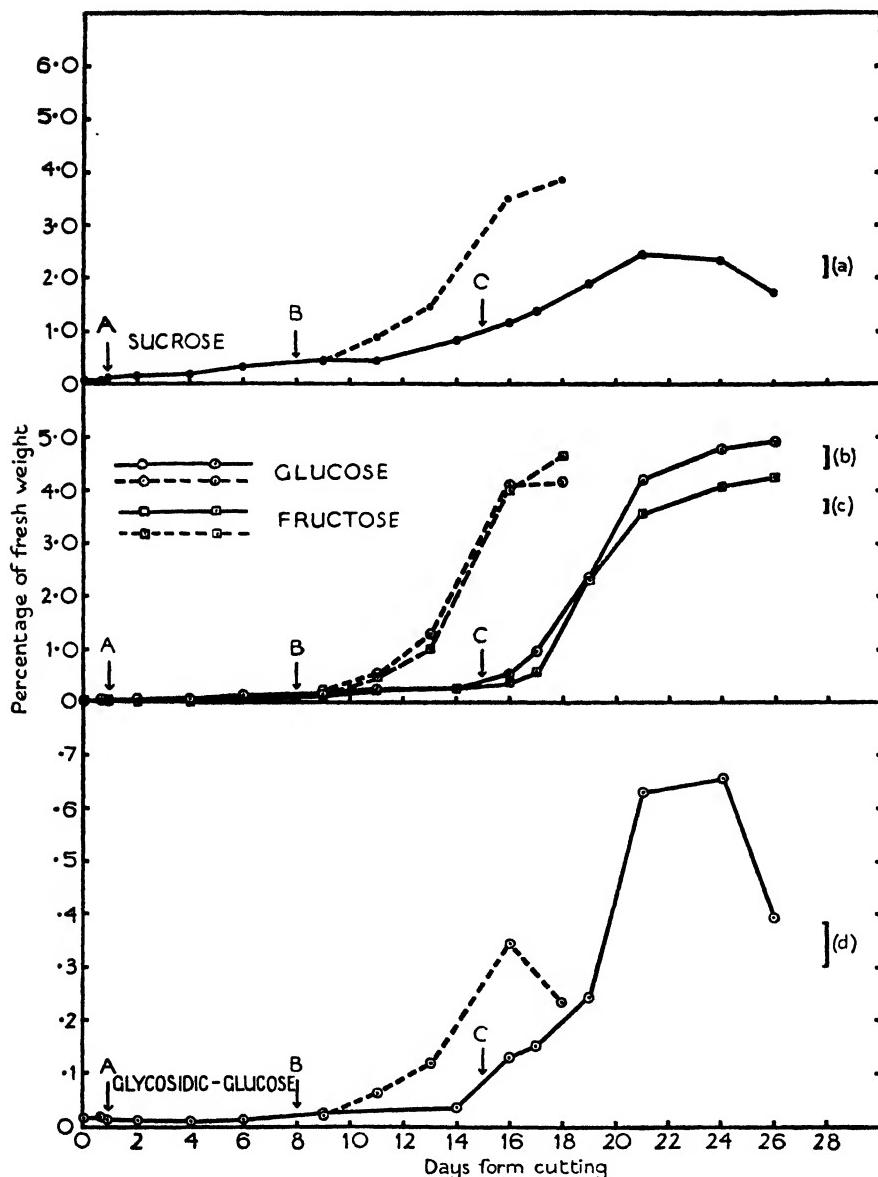
FIG. 4. Percentage amounts of sucrose, glucose, fructose, and of glycosidic-glucose in the pulps of 'heavy ¾-full' bananas during storage at 53° F. and ripening at 68° F. The vertical lines labelled (*a*), (*b*), (*c*), and (*d*) represent the minimum significant differences (*P* = 0·05) of (*a*) sucrose, (*b*) glucose, (*c*) fructose, and (*d*) glycosidic-glucose.

For a description of the ripening record of 'heavy ¾-full' fruit see the subscript to Fig. 2.

ripening room. As with sucrose, glucose, and fructose the increase in the glycosidic-glucose began in the long storage fruit before the transference of the fruit to the ripening room and continued during the early part of the ripening phase. Similar effects were observed in the heavy ¾-full fruit (Fig. 4). Previous analyses had indicated that glycosidic-glucose tended to be present in higher proportion in chilled than in unchilled ripened fruit, and this is clearly shown by the badly chilled long storage heavy ¾-full fruit shown in Fig. 4; the less severely chilled ¾-full fruit of Fig. 3 does not show this, but the margin of error in the glycoside estimations was such that the effect, if small, might not be detected.

VI. Percentage Amounts of Dry Matter and Various Carbohydrates in the Skin

The values obtained for the dry matter and carbohydrate percentages in the skin are set out for the ¾-full fruit in graphical form in Figs. 5 and 6, while the data for the heavy ¾-full fruit is summarized in Table III.

Table III

Percentage Amounts of various Carbohydrates in the Skins of heavy ¾-full Fruit during Storage and Ripening

Days from cutting.	Temp. (° F.).	Total dry wt.	Glucose.	Fruc- tose.	Sucrose.	Total sugars.	Starch.	Glyco- sidic- glucose.
	Room temp. (80–5)							
0	(80–5)	10·92	0·030	0·003	0·024	0·057	3·754	0·015
16 hr.	,,	11·07	0·044	0·003	0·047	0·094	3·566	0·020
23 hr.	53	11·20	0·054	0·004	0·053	0·111	3·460	0·011
2	,,	11·28	0·038	0·012	0·087	0·137	2·789	0·016
4	,,	10·70	0·022	0·005	0·115	0·142	2·363	0·009
6	,,	11·05	0·029	0·029	0·155	0·213	2·034	0·014
9	,,	10·74	0·081	0·029	0·199	0·309	2·475	0·019
11	,,	11·60	0·134	0·098	0·099	0·331	2·513	0·030
14	,,	11·19	0·175	0·062	0·217	0·454	2·386	0·028
16	68	11·93	0·185	0·099	0·183	0·467	2·186	0·087
17	,,	11·83	0·199	0·118	0·239	0·556	2·339	0·042
19	,,	11·87	0·341	0·256	0·502	1·099	1·737	0·056
21	,,	14·09	0·193	0·542	1·204	1·939	0·615	0·403
24	,,	14·01	0·176	0·571	0·945	1·692	0·288	0·436
26	,,	15·57	0·127	0·696	1·050	1·873	0·479	0·400
9	68	10·71	0·139	0·085	0·169	0·393	2·646	0·042
11	,,	11·18	0·193	0·163	0·466	0·822	2·451	0·034
13	,,	11·05	0·380	0·309	0·622	1·311	1·610	0·071
16	,,	11·30	0·725	0·564	0·468	1·757	0·471	0·287
18	,,	11·53	1·008	0·689	0·309	2·006	0·288	0·235
Sig. diff. (P = 0.05)		(0.46)	(0.060)	(0.045)	(0.066)	(0.128)	(0.332)	(0.063)

The upper part of the table gives data of fruits undergoing prolonged storage at 53° F. with subsequent ripening at 68° F., while the lower part gives the data for the ripening of fruit removed from cold storage after a shorter period.

Each value is the mean of two observations and estimates of the significant differences for the means are given at the foot of the columns.

(a) Total dry matter, starch, and total sugars.

The percentage of dry matter in the skin of ¾-full fruit increased slightly but steadily during the period of storage (Fig. 5), whereas in the corresponding pulp tissues dry matter slowly decreased in percentage (Fig. 1). The trends, though apparent, are not so clearly defined in the pulp (Fig. 2) and skin (Table III, column 3) of heavy ¾-full fruit. Water was, apparently, slowly lost from the skin to the pulp during the cold storage period. An increased rate of gain of dry matter percentage occurred in the skins of the long storage fruit (Fig. 5, continuous curve in upper portion) after the 17th day, while the values for the short storage fruit fluctuated somewhat after the transfer to 68° F. but eventually increased considerably between the 22nd and 28th days. The long-stored fruit after transference to 68° F. gave values for the dry matter content from the 25th to the 33rd day which indicated considerable water loss from the skin during ripening. The subject of water transference from skin to pulp during ripening has already received consideration (Barnell, 1941).

The starch and total sugar values for the skin were of much smaller magnitude than for the pulp but followed similar drifts. There was evidence of starch hydrolysis before the fruit was placed in cold storage (Fig. 5), and during the cold storage period there was, as in the pulp, some indication of resynthesis of starch shown by the skins of both ¾-full fruit (Fig. 5), and heavy ¾-full fruit (Table III, column 8). The rate of starch loss became considerably accelerated in the skins of the long storage fruit after the 14th day and, in fact, there was on day 18 little difference between the starch content of the skins of fruit which had remained in cold store until day 18 and those which had been transferred to the ripening room on the 15th day.

In the ripening room the starch content of the skins of fruit of both grades, both short- and long-stored, fell quickly to low values, there being no apparent difference in the rates for chilled and unchilled fruits.

The percentage amounts of the total sugars in the skins of ¾-full fruit (shown in Fig. 5), showed a steady increase during the storage period, with a more rapid rate of increase towards the end of the long storage period. When the short storage fruit was removed to 68° F. succeeding values of total sugar content of the skin showed a high rate of increase, starting abruptly from the time of change of temperature. A similar curve was obtained for the total sugars in the skins of the long-stored fruit after this was transferred to the ripening room (continuous total sugars curve in Fig. 5). Similar data were obtained for the skins of heavy ¾-full fruit (Table III, column 7).

The total sugars in the skins continued to increase in percentage amount until the fruit was black and falling from the stems. This continued increase in percentage amount was due to the high rate of water loss from the skin at this stage. All the drifts of sugar contents described in this section are partly obscured by the water content and dry matter drifts and will be more clearly

appreciated in section X, where the data are presented as total amounts per finger in the skin.
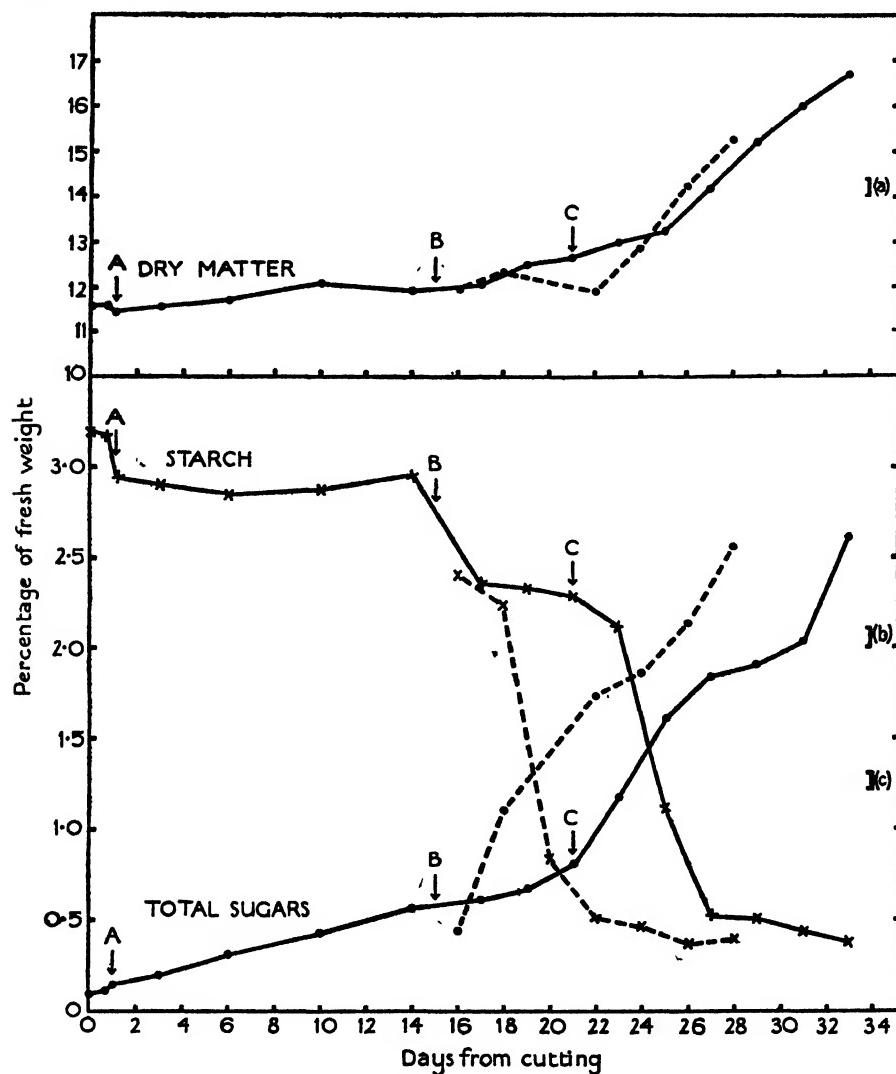
FIG. 5. Percentage amounts of dry matter, starch, and total sugars in the skins of '¾-full' bananas during storage at 53° F. and ripening at 68° F. The vertical lines labelled (a), (b), and (c) represent the minimum significant differences (P = 0·05) of (a) dry matter, (b) starch, and (c) total sugars.

For a description of the ripening record of '¾-full' fruit see the subscript to Fig. 1.

(b) Sucrose, glucose, fructose, and glycosidic-glucose.

Fig. 6 shows the drifts of the sugars and of glycosidic-glucose of the ¾-full fruit, while the data for the heavy ¾-full fruit are given in columns 4, 5, 6, and 9 of Table III. The percentage amounts of all the sugars were much lower
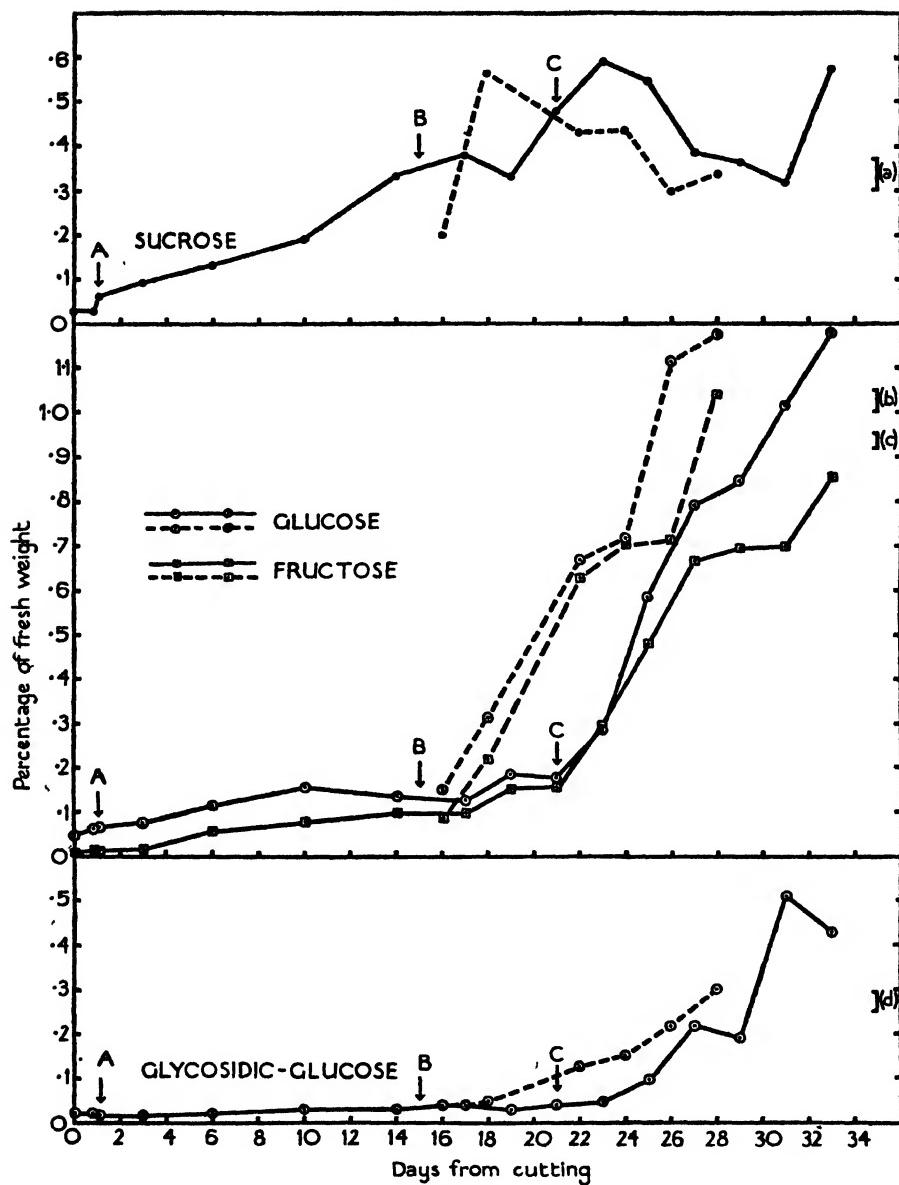
FIG. 6. Percentage amounts of sucrose, glucose, fructose, and of glycosidic-glucose in the skins of '¾-full' bananas during storage at 53° F. and ripening at 68° F. The vertical lines labelled (*a*), (*b*), (*c*), and (*d*) represent the minimum significant differences (*P* = 0·05) of (*a*) sucrose, (*b*) glucose, (*c*) fructose, and (*d*) glycosidic-glucose.

For a description of the ripening record of '¾-full' fruit see the subscript to Fig. 1.

than in the pulp. Sucrose was present in small amount in the skins of the freshly harvested fruit, but increased steadily through the storage period, rising rapidly to peak values within a few days of transference of the fruit to 68° F. in both the long and short storage fruit. As ripening proceeded the sucrose concentration fell during the over-ripe stage. The rise to the last observation was due mainly to the loss of water from the tissue at this time.

Glucose and fructose, with glucose in excess of fructose, increased slowly but steadily during the cold storage period, quickly rising in percentage amounts when the fruit was transferred to the ripening room. This effect held for both long- and short-stored fruit; the rising trends continued to the last sampling date.

Glycosidic-glucose slightly increased in percentage amount throughout the period of cold storage (data for ¾-full fruit in Fig. 6 and for heavy ¾-full fruit in column 9 of Table III). On transference to the ripening room the glycosidic-glucose in the skins of both ¾-full and heavy ¾-full fruit increased to higher values in the long storage than in the short storage fruit. One of the effects of chilling by prolonged cold storage therefore appears to be increased glycoside content in the skins as well as in the pulp (see V. *b*).

VII. Titratable Acid in Pulp and Skin

The very small titration values obtained from the aliquots of the diluted sub-sample of alcohol extract rendered the values for the titratable acid in pulp and skin unduly variable. The values for both grades of fruit are set out in Table IV as ml. N/10 NaOH per 100 gm. of tissue and, in spite of the variability, definite trends during storage and ripening are indicated. These trends are noted here, but further work is in progress on the acid metabolism of the banana in which pH determinations as well as more accurate determinations of titratable acid are being made.

From the data of Table IV it is seen that in general the pulp was slightly more acid than the skin throughout the storage and ripening period, this being true for both grades of fruit. There was a downward trend of the acidity in both grades during the period of cold storage in both skin and pulp but on removal to the ripening room increasing values were obtained for both long and short storage fruit, the values for the pulp in each instance increasing more rapidly than those of the skin, indicating that ripening takes place from the pulp outwards as in the fruit ripened under tropical conditions (Barnell, 1941). In the pulp and to a lesser extent in the skin the titratable acid attained higher values during ripening in long storage fruit than in short storage fruit, suggesting that a result of 'chilling' is increased acidity.

VIII. Percentage Amounts of Total Alcohol-soluble Substances in Pulp and Skin

Data recorded in Table V for the pulp and skin of the banana add further evidence of the presence in plant tissues of relatively large amounts of non-

TABLE IV. *Titratable Acid in Pulp and Skin (ml. N/10 NaOH per 100 gm. tissue)*

(a) ¾-full fruit

Days from cutting.	Temp. (° F.).	Titratable Acid. Pulp.	Skin.
0	Room temp. (80–5)	36·4	32·9
20 hr.	,,	31·6	32·9
26 hr.	53	32·8	25·6
3	,,	37·7	29·2
6	,,	35·2	28·0
10	,,	40·1	24·6
14	,,	30·8	29·5
17	,,	30·8	33·2
19	,,	36·9	25·7
21	68	34·1	33·0
23	,,	46·3	36·6
25	,,	54·8	48·9
27	,,	52·2	53·8
29	,,	74·1	55·1
31	,,	60·8	59·0
33	,,	59·7	60·2
16	68	33·2	26·8
18	,,	57·7	34·1
20	,,	55·3	—
22	,,	55·3	39·1
24	,,	55·2	47·7
26	,,	49·1	55·0
28	,,	51·5	59·9
	Sig. diff. (P = 0·05)	(10.6)	(5.4)

(b) Heavy ¾-full fruit

Days from cutting.	Temp. (° F.).	Pulp.	Skin.
0	Room temp. (80–5)	43·7	37·8
16 hr.	,,	53·2	32·9
23 hr.	53	52·4	32·9
2	,,	30·3	27·3
4	,,	21·7	15·4
6	,,	29·4	21·4
9	,,	28·9	24·0
11	,,	33·6	18·1
14	,,	43·3	27·6
16	68	40·8	39·2
17	,,	55·1	37·0
19	,,	66·0	39·2
21	,,	64·6	51·5
24	,,	61·0	61·5
26	,,	45·5	56·3
9	68	26·0	27·6
11	,,	40·6	31·2
13	,,	53·2	36·0
16	,,	58·2	45·9
18	,,	59·4	48·2
	Sig. diff. (P. = 0·05)	(8.7)	(7.4)

Each section of the table is subdivided, the upper portion containing values obtained during prolonged storage at 53° F. with subsequent ripening at 68° F. and the lower during ripening of fruit removed from cold storage after a shorter period. Each value is the mean of two observations. Estimates of the significant differences between the acid values are given at the foot of the third and fourth columns in both sections.

TABLE V. *Total alcohol-soluble Substances and total Sugars in Pulp and Skin*
(% of fresh weight)
(a) ¾-full fruit

		Pulp.		Skin.	
(1)	(2)	(3)	(4)	(5)	(6)
Days from cutting.	Temp. (° F.).	Total alcohol-soluble substances.	Total sugars.	Total alcohol-soluble substances.	Total sugars.
0	Room temp. (80–5)	1·782	0·205	1·852	0·086
20 hr.	,,	1·592	0·223	1·738	0·105
26 hr.	53	1·437	0·317	1·652	0·141
3	,,	2·167	0·472	1·812	0·188
6	,,	1·997	0·730	1·847	0·302
10	,,	2·522	0·927	2·222	0·423
14	,,	2·015	1·449	2·358	0·563
17	,,	2·378	1·953	2·495	0·601
19	,,	3·080	2·520	2·503	0·666
21	68	4·880	3·483	2·245	0·807
23	,,	7·972	6·085	3·163	1·171
25	,,	12·557	9·770	4·270	1·608
27	,,	14·825	12·235	5·142	1·836
29	,,	19·792	12·020	5·278	1·904
31	,,	17·760	11·250	5·667	2·029
33	,,	16·037	10·855	6·090	2·606
16	68	3·358	1·744	2·365	0·433
18	,,	8·955	5·871	3·212	1·097
20	,,	14·415	11·610	2·253	—
22	,,	15·703	12·720	4·010	1·728
24	,,	16·240	12·895	4·625	1·855
26	,,	16·310	13·285	5·435	2·125
28	,,	15·780	12·405	5·830	2·552

(b) Heavy ¾-full fruit

0	Room temp. (80–5)	1·153	0·097	0·947	0·057
16 hr.	,,	1·757	0·125	1·160	0·093
23 hr.	53	1·527	0·182	1·082	0·110
2	,,	1·042	0·225	1·352	0·137
4	,,	2·037	0·258	1·135	0·142
6	,,	1·321	0·559	1·445	0·213
9	,,	2·469	0·784	1·312	0·308
11	,,	3·162	0·959	1·900	0·331
14	,,	4·289	1·371	1·590	0·454
16	68	3·335	2·065	2·295	0·467
17	,,	4·693	2·910	2·095	0·556
19	,,	10·050	6·586	2·818	1·099
21	,,	13·632	10·210	5·428	1·940
24	,,	15·920	11·245	5·150	1·692
26	,,	16·030	10·855	5·210	1·873
9	68	1·975	0·849	1·377	0·393
11	,,	3·570	1·920	2·017	0·822
13	,,	6·260	3·759	2·820	1·311
16	,,	13·740	11·515	4·393	1·757
18	,,	15·260	12·630	3·855	2·006

In each section of the table the upper portion contains values obtained during prolonged storage at 53° F. with subsequent ripening at 68° F., and the lower from fruit removed from cold storage after a shorter period. Each value is the mean of two observations.

sugar substances in the alcohol extract. The importance and significance of this non-sugar fraction has been discussed previously (Barnell, 1940, 1941), but it may be mentioned that this fraction contains, amongst other substances, reducing compounds to which many reagents used for sugar estimations are sensitive (potassium ferricyanide in particular). Values for the total alcohol-soluble substances should obviously exceed the corresponding values obtained for total sugars unless the non-sugars present have reducing powers higher than that of glucose for the reagents used in estimation. Some investigators have found the percentage of total sugars in the ripe pulp of the Gros Michel banana to be as high as 26 per cent. (Leuscher, 1902), while several give values of 19–20 per cent. (Reich, 1911; Wolfe, 1931; Banana Ripening Manual, 1937).

The highest value given in Table V for the total alcohol-soluble substances in the ripened pulp is 19·79 per cent., while the majority of values for ripe fruit lie between 13 and 19 per cent. Even allowing for the probably incomplete extraction of all alcohol-soluble substances (as duration of extraction was standardized for sugar extraction only), it would seem that either the material used by various workers has been very different from that used in this work or that the methods have, in some instances, given very inflated values for the total sugar content.

The drifts of the percentage amounts of total alcohol-soluble substances in both pulp and skin followed similar courses to those of the total sugars, though the proportion of sugars in the total varied considerably. In the unripe green fruit, as received, and during the early days of storage the proportion of sugars in the extract was very low (approximately 10 per cent.), but as the fruit ripened at 68° F. the proportion increased to approximately 70 per cent. of the total alcohol-soluble substances.

There is no apparent difference in the drifts of amounts of total alcohol-soluble substances in the pulp and skin between the ¾-full and the heavy ¾-full fruit; no difference was observed between the values for the pulp of chilled and unchilled fruit, but values for the total alcohol-soluble substances tended to be higher in the skins of chilled (long storage) fruit than in unchilled fruit of both grades.

IX. Changes in the Total Amounts per Finger of Dry Matter and Various Carbohydrates in the Pulp

The advantages, when an adequate sampling technique has been employed, of expressing the data for the composition of the fruit as total amounts of each substance per single finger, in the pulp and in the skin have been discussed in an earlier communication (Barnell, 1941). This method has again been adopted so that the actual gains or losses of the amounts of the estimated carbohydrates may be followed without the distortion produced by underlying drifts of water content. In this section accordingly, the data for the pulp are presented as amounts per single finger in the pulp, while in the next
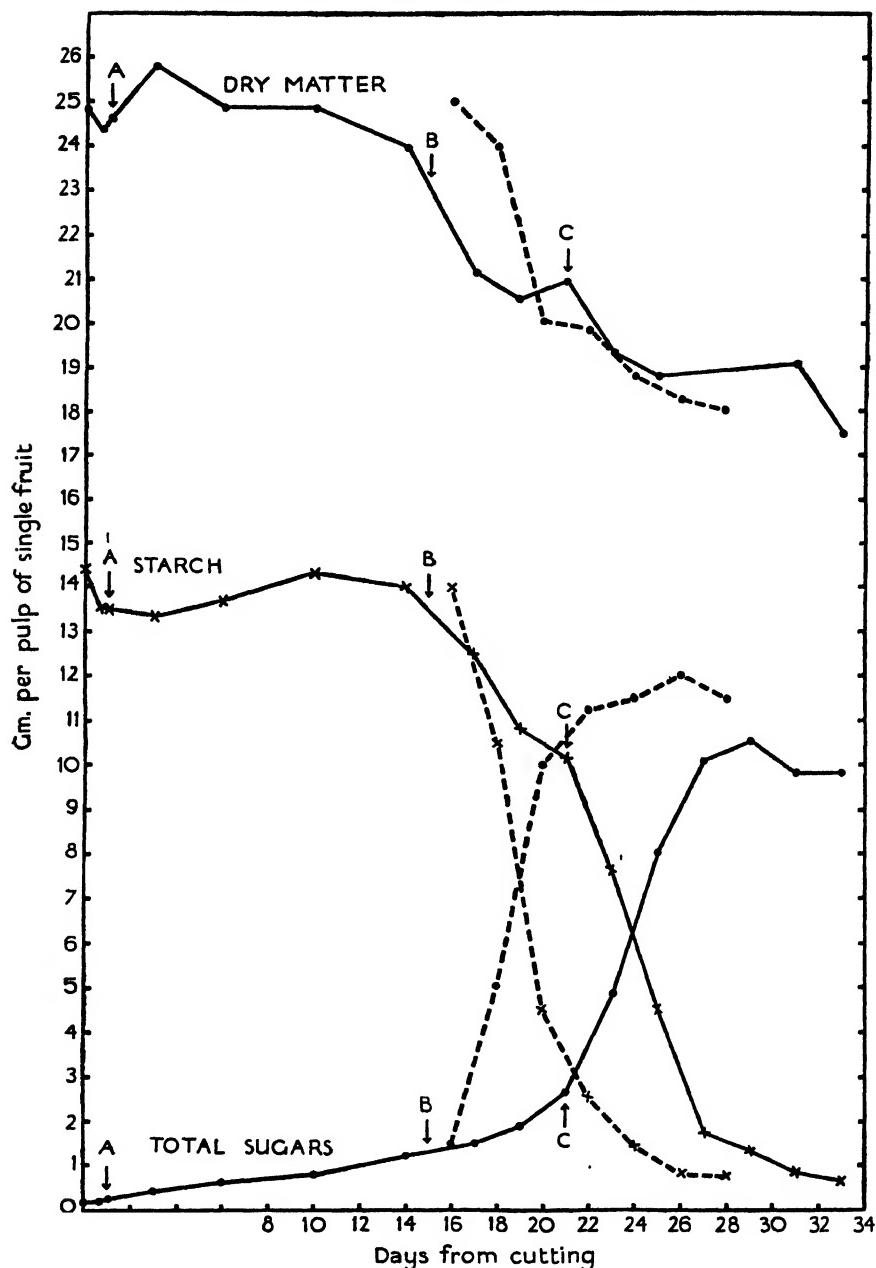
FIG. 7. Amounts of dry matter, starch, and total sugars in the pulps of '¾-full' bananas, given as gm. per pulp of a single fruit during storage at 53° F. and ripening at 68° F.
For a description of the ripening record of '¾-full' fruit see the subscript to Fig. 1.

section the skin data are given as amounts per single finger present in the skin.

(a) Total dry matter, starch, and total sugars.

Figs. 7 and 8 show the changes which occurred in the total amounts of dry matter, starch, and total sugars in the pulps of ¾-full and heavy ¾-full fruit respectively during storage and subsequent ripening. The dry matter in the pulp of both grades of fruit, though fluctuating in amount somewhat, showed a small but consistent rate of loss during the earlier stages of storage. The drop in the total amount of dry matter in the long storage fruit observed between 14 and 17 days of storage in the ¾-full fruit (Fig. 7), and between 11 and 14 days in the heavy ¾-full fruit (Fig. 8), was partly due to the sampling change and partly to the increased rate of loss as ripening began in the late storage period; a higher respiration rate was also, at least partly, responsible. The high rate of loss of dry matter continued when the fruit was placed in the ripening rooms.

The short storage fruit of both grades was sampled only from upper-row fingers of the 3rd hand, and the curves for the total dry matter of the pulp during the ripening period at 68° F. (discontinuous curves in upper portions of Figs. 7 and 8) fall throughout the period, with maximum rate of loss of dry matter during the yellowing to full yellow or eating-ripe stages of the fruit.

A second peak rate of total dry matter loss was a feature of the late stages of senescence of the pulp of fruit ripened at tropical temperatures (Barnell, 1941); this was not observed in the ripening of either long- or short-stored fruit but the high rate of loss was considerably prolonged owing to the lower temperature of ripening (68° F. as opposed to 80 to 85° F.).

The lower portions of Figs. 7 and 8 contain the data for the total amounts of starch and total sugars per finger in the pulps of ¾-full and heavy ¾-full fruit respectively. Some loss of starch was observed within the 1st day in the ¾-full fruit and within the first 2 days in the heavy ¾-full fruit, but during the cold storage period a possible small resynthesis of starch was again suggested in both grades of fruit (compare V. *a*); the origin of the materials for this resynthesis did not appear to be the estimated sugars, as their accumulation was steady and consistent throughout the storage period. The starch in the pulps of the short-stored fruit fell in amount on removal of the fruit to the ripening room, reaching the minimum value within 10 days for both grades of fruit. Falling values for the starch amounts occurred in the long-stored fruit of both grades whilst still at 53° F. and little sign of change in the rate of loss of starch was observed when the fruit was transferred to the ripening room.

Total sugars followed, in general, an inverse trend to that of starch. But the final amount of sugars accumulated did not in any instance equal the initial amount of starch present. In both grades of fruit the final amount of

total sugars present in the pulp of the ripened fruit was greater in the short-stored than in the long-stored fruit (Figs. 7 and 8). The discrepancy between starch loss and sugar gain may be ascribed to loss of carbohydrate in respira-
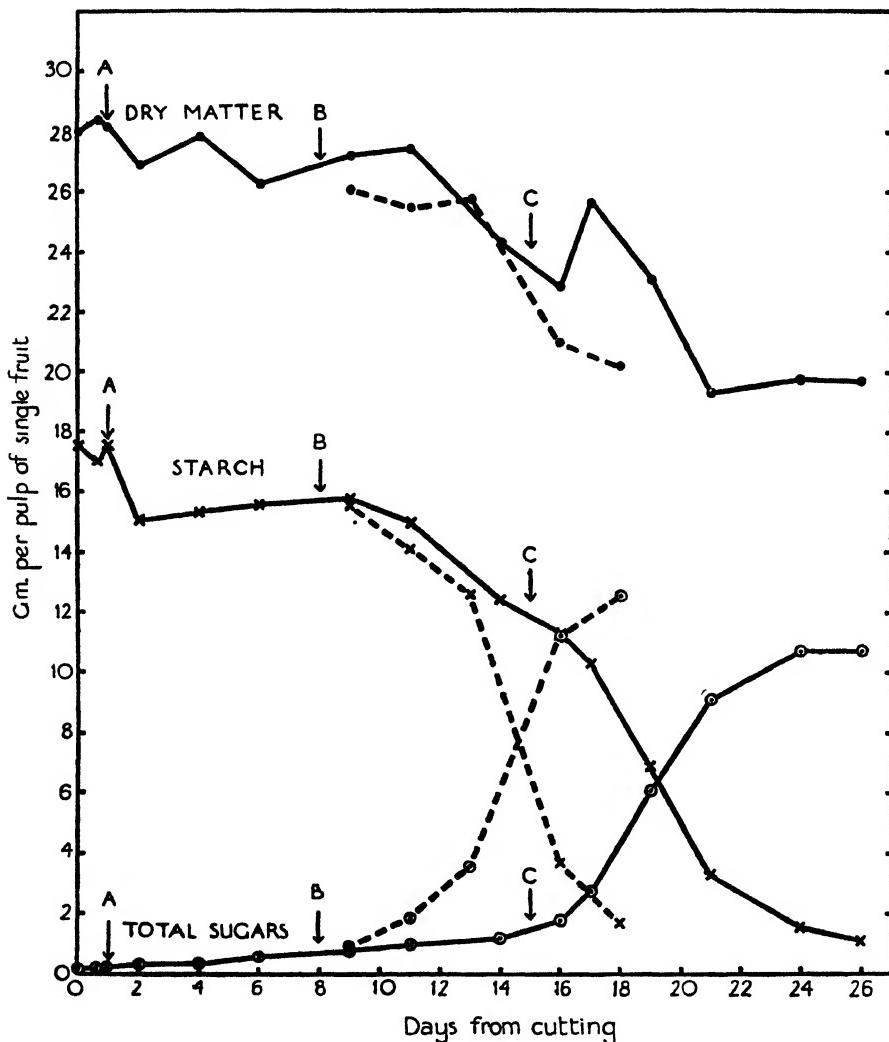
FIG. 8. Amounts of dry matter, starch, and total sugars in the pulp of 'heavy ¾-full' bananas, given as gm. per pulp of a single fruit during storage at 53° F. and ripening at 68° F.

For a description of the ripening record of 'heavy ¾-full' fruit see the subscript to Fig. 2.

tion and the difference in final sugar contents of the long- and short-stored fruits to the extended period of respiration undergone by the long-stored fruit.

The general drifts of total dry matter, starch, and total sugars in Figs. 1 and 7 for ¾-full and Figs. 2 and 8 for heavy ¾-full fruit, are seen to be very similar on either a percentage or total amount basis.

(b) Sucrose, glucose, fructose, and glycosidic-glucose.

Curves for the sugars in the pulp are given in Figs. 9 and 10 for the ½-full and heavy ¾-full fruit respectively.

Sucrose accumulated more rapidly than the reducing sugars during the
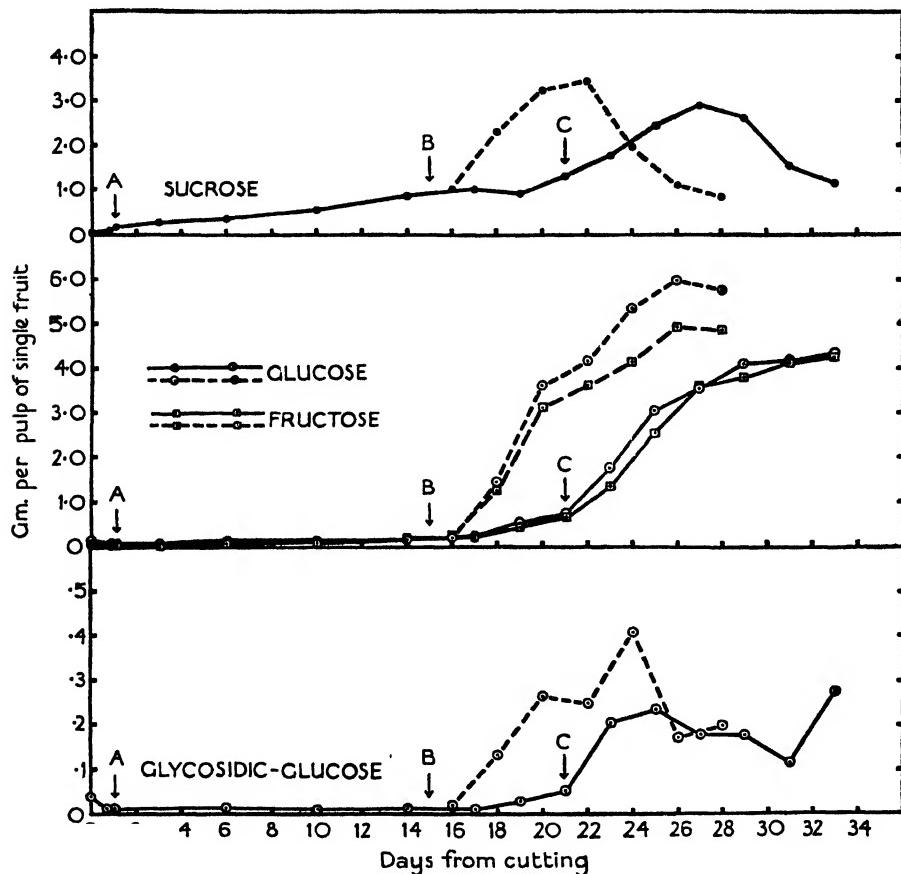
FIG. 9. Amounts of sucrose, glucose, fructose, and of glycosidic-glucose in the pulps of '½-full' bananas, given as gm. per pulp of a single fruit during storage at 53° F. and ripening at 68° F.

For a description of the ripening record of '½-full' fruit see the subscript to Fig. 1.

early stages of storage, the ¾-full grade showing a very definite increase during the first 24 hours. Accumulation continued until the fruit was yellow-ripe, then losses were shown in all instances except by the short storage heavy ¾-full fruit for which, however, samples were not obtained during the over-ripe phase. The total amount of sucrose at the peak value was higher in the short-stored than in the long-stored fruit in each instance, i.e. the chilled fruit had a smaller sucrose content in the pulp than the unchilled.

Glucose and fructose remained low in the pulp during the cold storage

periods; when the short storage fruit of both grades was transferred to the ripening room accumulation of the reducing sugars took place much more rapidly, glucose increasing in amount to a greater extent than fructose, particularly during the phase of most rapid increase of the two sugars. The long storage fruit of both grades showed acceleration of the accumulation rates of glucose and fructose during the last 4 or 5 days of cold storage, the upward trend being continued more rapidly when the fruit was transferred to the ripening room.

Glycosidic-glucose showed little increase during the cold storage periods apart from a small rise towards the end of the long storage period with each grade. In the ripening room the glycosidic content of the pulp rose to relatively high values during the eating-ripe stage, afterwards usually falling in amount. In the heavy ¾-full fruit more glycosidic-glucose accumulated in the long-stored, chilled fruit than in the unchilled fruit.

The general drifts of dry matter and estimated carbohydrates in the pulp on either a percentage or total amount basis are seen to be similar in both ¾-full fruit (Figs. 3 and 9), and heavy ¾-full fruit (Figs. 4 and 10). This is in agreement with the corresponding data for fruit at tropical temperatures (Barnell, 1941).

X. Changes in Total Amounts per Finger of Dry Matter and Various Carbohydrates in the Skin

(a) *Total dry matter, starch, and total sugars.*

The data for the total dry matter, total sugars, and starch in the skins of the ¾-full fruit are given in Fig. 11 and for the heavy ¾-full fruit in columns 3, 7, and 8 of Table VI. Sampling variation, and particularly the transition from upper-row fingers to those of the lower row of the 3rd hand, render the dry matter observations suitable for indicating trends but not for calculating rates of utilization in respiration (Barnell, 1941). In both grades of fruit little change in the total dry matter per finger in the skin could be detected during the early stages of the cold storage period. A considerable fall in the amount occurred in the long storage fruit towards the end of the storage period in each instance: this is distorted by the sampling changes between the 14th and 17th days in the ¾-full fruit and between the 11th and 14th days in the heavy ¾-full fruit. The short storage fruit of both grades on removal to the ripening room showed, after initial fluctuations, a rapid fall in the dry matter of the skin during the late yellowing to eating-ripe stages. If the rapid fall in dry matter is regarded as an indication of ripening, the skin was later than the pulp in starting the accelerated ripening process by approximately 2 days for the long storage ¾-full fruit (Figs. 7 and 11). The difference was not so evident in the heavy ¾-full fruit where the sampling transition confused the comparison.

The total amount of starch per finger in the skin fell in both grades during the 1st day and then showed little change during the early days of storage; an
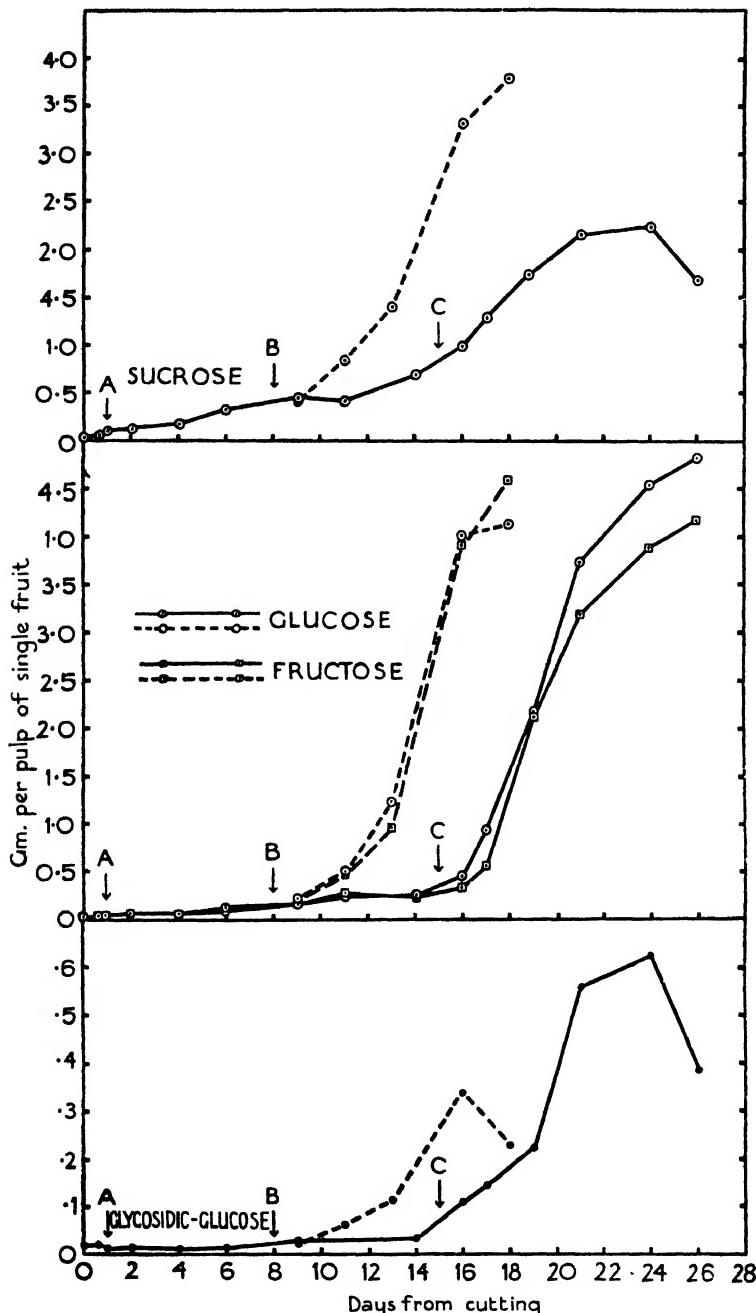
FIG. 10. Amounts of sucrose, glucose, fructose, and of glycosidic-glucose in the pulps of 'heavy ¾-full' bananas, given as gm. per pulp of a single fruit during storage at 53° F. and ripening at 68° F.

For a description of the ripening record of 'heavy ¾-full' fruit see the subscript to Fig. 2.

accelerated rate of fall, exaggerated by the sampling change, occurred in the ¾-full fruit between the 14th and 17th days and continued more rapidly when the fruit was placed in the ripening room, low values being obtained for the

TABLE VI

Amounts (per finger) of various Carbohydrates in the Skins of heavy ¾-full Fruit during Storage and Ripening

Days from cutting.	Temp. (° F.).	Total dry matter. (gm.).	Glucose. (gm.).	Fruc-tose. (gm.).	Sucrose. (gm.).	Total sugars. (gm.).	Starch. (gm.).	Glyco-sidic-glucose.
0	Room (80–5)	6·86	0·019	0·002	0·015	0·036	2·354	0·009
16 hr.	,,	6·89	0·027	0·002	0·029	0·058	2·223	0·012
23 hr.	53	6·94	0·034	0·002	0·033	0·069	2·146	0·007
2	,,	6·78	0·023	0·007	0·052	0·082	1·679	0·010
4	,,	6·47	0·013	0·003	0·070	0·086	1·431	0·005
6	,,	6·65	0·017	0·017	0·093	0·127	1·223	0·008
9	,,	6·33	0·048	0·017	0·117	0·182	1·459	0·011
11	,,	6·85	0·079	0·058	0·058	0·195	1·481	0·018
14	,,	*5·36	0·084	0·030	0·104	0·218	1·145	0·013
16	68	5·56	0·086	0·046	0·085	0·217	1·109	0·040
17	,,	6·64	0·112	0·066	0·134	0·312	1·310	0·024
19	,,	6·21	0·178	0·134	0·263	0·575	0·908	0·029
21	,,	6·08	0·083	0·234	0·520	0·837	0·266	0·174
24	,,	5·44	0·068	0·221	0·366	0·655	0·112	0·169
26	,,	5·48	0·045	0·246	0·370	0·661	0·169	0·141
9	68	6·25	0·089	0·050	0·099	0·238	1·543	0·025
11	,,	6·51	0·113	0·096	0·274	0·483	1·441	0·020
13	,,	6·21	0·214	0·174	0·349	0·737	0·904	0·040
16	,,	5·62	0·360	0·280	0·233	0·873	0·234	0·143
18	,,	4·84	0·463	0·316	0·142	0·921	0·132	0·108

The data of this table are derived from those of Table III and from column 9 of Table I, the values being recalculated as total amounts of the estimated substances in the skin of a single finger. The single finger weight is the mean of the 30 (29 or 28 in some instances) fingers used in each sampling.

* The asterisk indicates that the values for that row and those below it within the same section (long storage) were obtained from fingers of the lower rows of the 3rd hands of the bunches sampled; preceding values were obtained from upper-row fingers.

ripe and over-ripe fruit. The starch in the skin of the short storage fruit fell in total amount from the time of transfer of the fruit to the ripening room to the last sampling date when the fruit was over-ripe.

Total sugars in both long and short storage fruit of both grades increased in total amount in the skin as the starch decreased, but the final sugar values were not comparable in magnitude with the starch values of the unripe fruit.

The general drifts of starch and total sugars in the skin of ¾-full fruit are seen to be approximately similar in both percentage and total amount bases (Figs. 5 and 11), though at 68° F. the decrease in starch and increase in total sugars on the basis of total amount per single finger were less marked than on
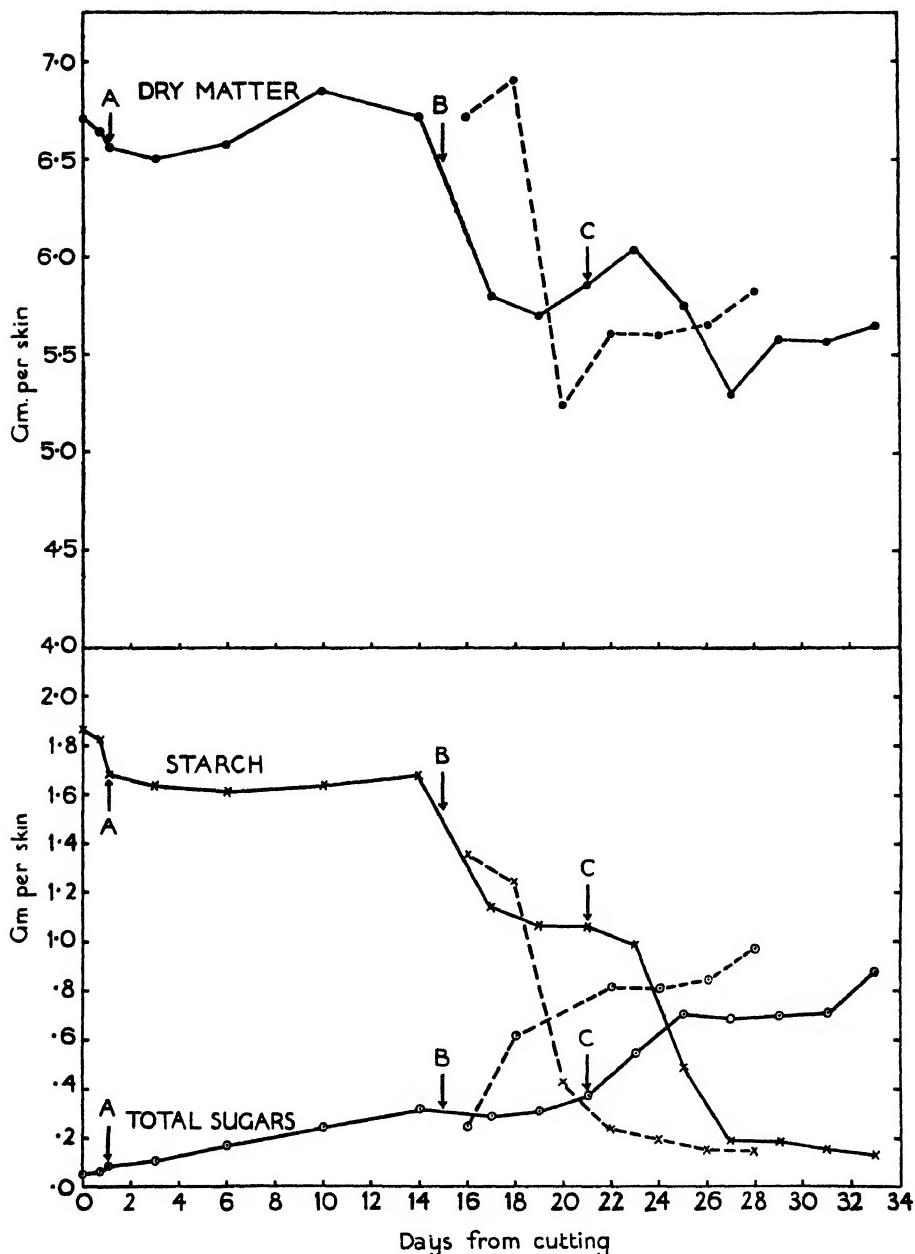
FIG. 11. Amounts of dry matter, starch, and total sugars in the skins of '¾-full' bananas, given as gm. per skin of a single fruit during storage at 53° F. and ripening at 68° F.

For a description of the ripening record of '¾-full' fruit see the subscript to Fig. 1.

the percentage basis. But the dry matter showed opposite drifts on the two bases of expression due to the very considerable loss of water from the skin.

(b) Sucrose, glucose, fructose, and glycosidic-glucose.

The sucrose in the skins of the fruit of each grade (Fig. 12 and Table VI,
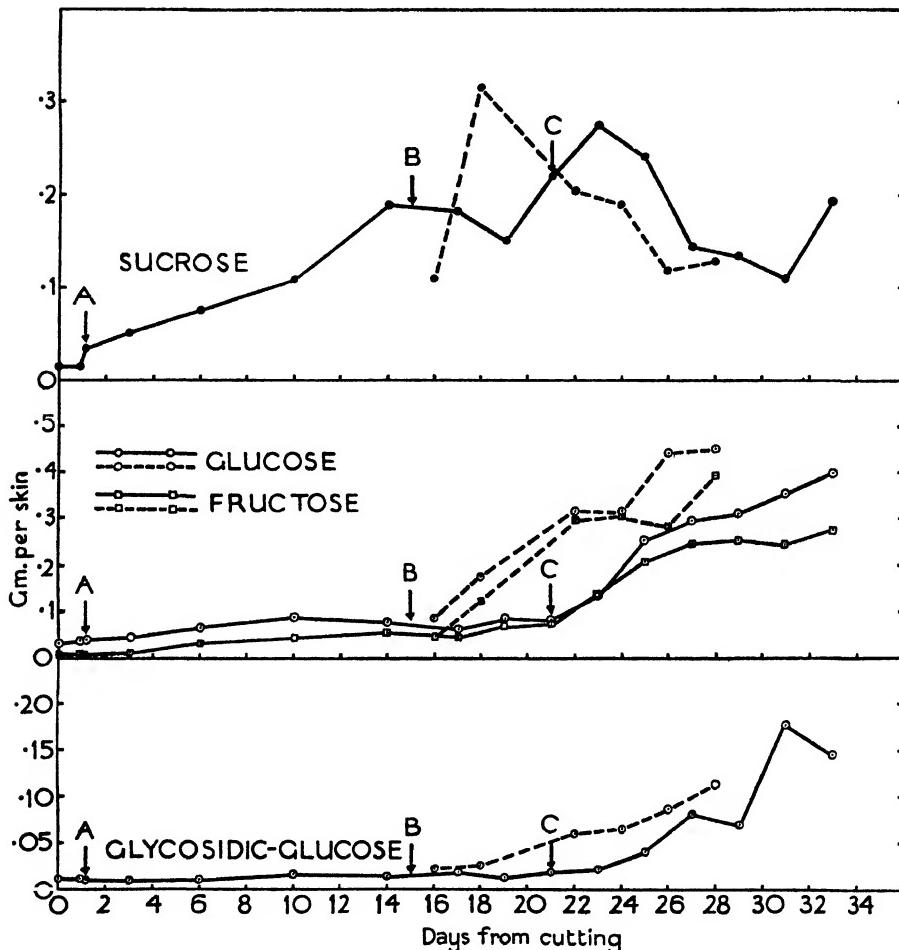
FIG. 12. Amounts of sucrose, glucose, fructose, and of glycosidic-glucose in the skins of '¾-full' bananas, given as gm. per skin during storage at 53° F. and ripening at 68° F.

For a description of the ripening record of '¾-full' fruit see the subscript to Fig. 1.

column 6), increased in amount more than the reducing sugars during the storage periods, eventually attaining maximum values while the fruit was in the ripening room and then decreasing. In both the long- and the short-stored fruit the maximum amount of sucrose in the skin was reached 3 to 5 days earlier than the corresponding maximum in the pulp, i.e. whilst the skin was yellowing; by the full yellow stage when the pulp was ripe and had

maximum sucrose present the amount in the skin in each instance was decreasing.

Glucose and fructose, shown in the middle portion of Fig. 12 for ¾-full fruit and in columns 4 and 5 of Table VI for heavy ¾-full fruit, increased in amount in the skins to a lesser extent than sucrose during the cold storage periods. Glucose was in excess of fructose throughout the storage and ripening periods except during the later stages of the ripening of the long-stored heavy ¾-full fruit when low values for glucose and relatively high values for fructose amounts were obtained. The implications of this observation in relation to chilling remain to be further investigated.

The drift of the glycosidic-glucose content of the skins of both grades of fruit (Fig. 6, bottom, and Table VI, column 9), showed little increase during the cold storage periods but considerable increases during the ripening stages at 68° F. Higher values were recorded for both grades in the fruit which received the longer period of cold storage, and were therefore chilled, than in the fruit receiving the shorter period.

The drifts of sucrose and the reducing sugars and glycosidic-glucose in ¾-full fruit showed similar trends on the basis of either percentage or total amount (Figs. 6 and 12).

XI. Discussion

(a) Present banana storage practices and the basic problems for improvement.

The present procedure in the refrigerated overseas transport of bananas consists in (i) cutting fruit of suitable 'grade', (ii) transporting it at a temperature sufficiently low to retard but not to cause a marked diversion from the normal ripening process, and (iii) ripening at temperatures known from experience to give an acceptably palatable fruit. It is essentially a prolongation of the pre-climacteric phase of comparatively immature fruit; ¾-full fruit can remain in air at the storage temperature now used for 12 to 20 days while heavier grades of fruit destined for less distant markets can be carried in storage for shorter periods without incurring the functional disease of 'chilling'.

A central economic problem, then, is to devise storage methods permitting the carriage of heavier grades of fruit—a possible 50 per cent. increase in weight appears maximal (Wardlaw, Leonard, and Barnell, 1939 *a*)—to existing markets and of prolonging the storage period of the present grades to increase the margin of safety. A further problem is to explore possible modifications to improve the quality of the ripe fruit and to reduce wastage during the ripening period.

The view is held that, in common with many practical problems, the mode of approach most likely to lead to success is the fundamental one, and accordingly the metabolism of the banana fruit has been, and is being, studied, both on the plant and during storage and ripening from broad physiological and biochemical aspects.

(b) *Carbohydrate chemistry of the banana during storage and ripening, with particular reference to 'chilling'.*

The course of the ripening processes as indicated by carbohydrate changes has already been followed in the pulp and skin of Gros Michel bananas held under tropical conditions (Barnell, 1941). The present work is concerned with the carbohydrate chemistry during storage and ripening of fruits which were cut at two different commercial grades, and (a) given cold storage and ripening treatments such as would produce fruit satisfactory to the present markets, and (b) given cold storage sufficiently prolonged to produce 'chilled' fruit. One object has been to determine whether storage at 53° F. delays ripening simply by slowing down all the metabolic processes coincidently and uniformly or whether a diversion occurs from the 'normal' ripening observed at tropical temperatures. The fact that readily recognizable symptoms of 'chilling' are developed (Wardlaw and McGuire, 1931) suggested that a diversion might be expected. An investigation of the 'chilling' process was therefore included to ascertain, if possible, the nature and extent of such diversions.

In their main outline the carbohydrate changes which occur during the ripening of the banana pulp at temperatures above that producing 'chilling' are well established. The large reserve of starch present in the unripe fruit decreases during ripening, while sugars increase; sucrose accumulates primarily but after reaching a maximal value declines, while glucose and fructose increase, in approximately equal amounts, at first somewhat slowly, but very rapidly after the sucrose peak has been attained.

That sucrose increases when starch decreases in plant storage tissues has been observed in the potato by de Wolff (1926) and in apple tissue by Onslow, Kidd, and West (1932) while the reverse effect—the accumulation of sucrose preceding the condensation of starch—has been observed in wheat ears by Barnell (1936). In certain instances the sequence of reactions involved in starch hydrolysis within living plant tissue is, apparently: starch → hydrolysis products → sucrose → invert sugar, the actual mechanism of sucrose formation being undefined. The reducing sugars accumulate within the banana tissue mainly as invert sugar as though derived from inversion of sucrose by the very active sucrase which the banana contains (Mieran, 1893).

If this sequence of reactions is accepted the concentration of sucrose corresponding to any particular stage of starch hydrolysis (or fruit ripeness) will depend on the relative velocities of sucrose formation and sucrose hydrolysis which in turn will depend on the activities of the respective enzyme complex for each of the reactions. If these enzyme complexes are differentially sensitive to the external conditions of the fruit, we may anticipate differing relations between the sucrose and reducing sugar concentrations in fruits which have been subjected to different treatments. This may, in part, help to explain the widely differing results obtained by different investigators in different parts of the world.

The present investigation has shown a quantitative effect of grade of fruit and of storage treatment on the subsequent starch hydrolysis rate. The middle part of the starch curves (percentage fresh weight data, Figs. 1 and 2) for the ripening pulp at 68° F. is logarithmic in form, a straight line resulting when the logarithm of the starch percentage is plotted against time; the slope of this line, K (negative), has been obtained for each of the grades and for the long and short storage fruit in each instance and is presented below in Table VII together with a value obtained from heavy ¾-full fruit ripened at

TABLE VII

Rate of Loss of Starch from Pulp

Grade.	Days at 53° F.	Ripening temperature.	K (index of starch loss rate).
Heavy ¾-full	0	Room (80–5° F.)	0·250*
,,	7	68° F.	0·180
,,	14	,,	0·135
¾-full	14	,,	0·145
,,	20	,,	0·130

* The value for K for the pulp of heavy ¾-full bananas ripened under tropical conditions was obtained from the data given in a previous publication (Barnell, 1941).

tropical temperatures (Barnell, 1941). The heavy grade of fruit ripened under tropical conditions had the greatest rate of starch loss, while, within each grade of fruit ripening at 68° F. after short and long cold storage, that receiving the shorter period subsequently lost starch more rapidly than that receiving the longer period. There is also the indication that the heavier grade lost starch during ripening more rapidly than the thinner grade, though since the two grades did not receive identical treatments this cannot be definitely stated: however, very thin grades of fruit will not ripen under the most favourable conditions. Extreme chilling experienced by fruit left for long periods at 53° F. results in very slow and abnormal ripening (Leonard and Wardlaw, 1941). The enzyme complex concerned in starch hydrolysis in the pulp is apparently very sensitive to the temperature and duration of storage.

(c) The 'eating-ripe' banana and the effects of 'chilling' on composition and quality.

Different storage and ripening treatments produce fruits of different eating quality, and from the data now available it is possible to formulate to some extent the chemistry of quality differences, at least as regards the major carbohydrates estimated. Fruit which has been ripened under tropical conditions has a less attractive texture and 'flatter' taste than fruit which has received short cold storage treatment followed by ripening at 68° F. Prolonged cold storage lends an undesirable appearance to the fruit on subsequent ripening, the skin being to a greater or less extent, russet-coloured with a 'sooty' or 'smoked' appearance beneath the yellow; the pulp is rather astringent to the palate.

Collated data are given in Tables VIII and IX for selected constituents of the pulp and skin of the two grades of fruit in the 'eating-ripe' condition as indicated by skin colour after the various treatments shown. From the tables

TABLE VIII

Quality of Bananas. Carbohydrate Composition of the Pulps of chilled and of 'normal' Fruits and of Fruits ripened under tropical Conditions, during the 'eating-ripe' Stage. Starch, &c., expressed as percentage of fresh weight; acid as ml. N/10 NaOH per 100 gm. fresh wt.

	Heavy ¾-full.	Heavy ¾-full.	Heavy ¾-full.	Standard ¾-full.	Standard ¾-full.
	Ripened at trop. temp.	7 days at 53° F., ripened at 68° F.	14 days at 53° F., ripened at 68° F.	14 days at 53° F., ripened at 68° F.	20 days at 53° F., ripened at 68° F.
Eating quality.	'Boiled.'	Excellent.	'Chilled.'	Excellent.	'Chilled.
Starch	(7) 1·56	(16) 3·74	(19) 7·52	(20) 5·25	(25) 5·49
	(9) 0·654	(18) 1·71	(21) 3·67	(22) 2·89	(27) 2·13
	(11) 0·357	—	(24) *1·62	(24) *1·61	(29) 1·51
Total sugars	(7) 13·450	(16) 11·515	(19) 6·586	(20) 11·610	(25) 9·770
	(9) 12·770	(18) 12·630	(21) 10·210	(22) 12·720	(27) 12·235
	(11) 12·600	—	(24) *11·245	(24) *12·895	(29) 12·020
Sucrose	(7) 3·660	(16) 3·390	(19) 1·888	(20) 3·755	(25) 2·950
	(9) 1·700	(18) 3·820	(21) 2·421	(22) 3·890	(27) 3·510
	(11) 0·990	—	(24) *2·356	(24) *2·220	(29) 2·970
Glycosidic-glucose	(7) 0·344	(16) 0·347	(19) 0·241	(20) 0·307	(25) 0·282
	(9) 0·227	(18) 0·232	(21) 0·630	(22) 0·280	(27) 0·213
	(11) 0·209	—	(24) *0·656	(24) *0·235	(29) 0·201
Acid	(7) 65·0	(16) 58·2	(19) 66·0	(20) 55·3	(25) 54·8
	(9) 64·9	(18) 59·4	(21) 64·6	(22) 55·3	(27) 52·2
	(11) 51·7	—	(24) *61·0	(24) *55·2	(29) 74·1

A range of values is given of each substance for each grade and treatment extending through the eating-ripe stage. The values marked * are for fruit which would be judged by eye to be slightly over-ripe. Values for heavy ¾-full fruit ripened under tropical conditions were obtained from the data of an earlier publication (Barnell, 1941). The figures in brackets indicate the time in days from cutting.

the range of values for the composition of pulp and skin within the 'eating-ripe' stage of fruit receiving one treatment may be compared with those receiving other treatments.

The decelerating effect produced by chilling on the rate of starch hydrolysis results in the starch content of pulp and skin of chilled fruit being generally higher and total sugars generally lower than in unchilled fruit at stages comparable on the basis of appearance (rows 3 and 4 in Tables VIII and IX); starch is present in least percentage amount in the fruit ripened under tropical conditions. Glycosidic-glucose is, on the whole, present in greater percentage amount in 'chilled' fruit, in both pulp and skin than in unchilled; this glucose may be derived in part from tannins which have been found to be an important quality factor in that the astringency of chilled fruit

is due, in part at least, to their relatively high 'free' tannin content (H. R. Barnell, unpublished data). Titratable acid also occurs to a greater extent in the pulp and skin of 'ripe' chilled fruit than in unchilled fruit but is also

TABLE IX

Quality of Bananas. Composition of the Skins *of chilled and 'normal' Fruits ripened under tropical Conditions, during the 'eating-ripe' Stage. Data expressed as in Table VIII*

Grade of fruit.	Heavy ¾-full.	Heavy ¾-full. 7 days at 53° F., ripened at 68° F.	Heavy ¾-full. 14 days at 53° F., ripened at 68° F.	Standard ¾-full. 14 days at 53° F., ripened at 68° F.	Standard ¾-full. 20 days at 53° F., ripened at 68° F.
Treatment.	Ripened at trop. temp.				
Eating quality.	'Boiled.'	Excellent.	'Chilled.'	Excellent.	'Chilled.'
Starch	(7) 0·480	(16) 0·471	(19) 1·737	(20) 0·836	(25) 1·119
	(9) 0·266	(18) 0·288	(21) 0·615	(22) 0·504	(27) 0·513
	(11) 0·426	—	(24) *0·288	(24) *0·456	(29) 0·502
Total sugars	(7) 2·105	(16) 1·757	(19) 1·099	(20) —	(25) 1·608
	(9) 2·521	(18) 2·006	(21) 1·940	(22) 1·728	(27) 1·836
	(11) 3·379	—	(24) *1·692	(24) *1·855	(29) 1·908
Sucrose	(7) 0·585	(16) 0·468	(19) 0·502	(20) —	(25) 0·546
	(9) 0·762	(18) 0·309	(21) 1·204	(22) 0·432	(27) 0·384
	(11) 0·849	—	(24) 0·945	(24) 0·438	(29) 0·365
Glycosidic-glucose	(7) 0·182	(16) 0·287	(19) 0·056	(20) —	(25) 0·095
	(9) 0·273	(18) 0·235	(21) 0·403	(22) 0·128	(27) 0·219
	(11) 0·367	—	(24) *0·436	(24) *0·150	(29) 0·191
Acid	(7) 39·0	(16) 45·9	(19) 39·2	(20) —	(25) 48·9
	(9) 57·9	(18) 48·2	(21) 51·5	(22) 39·1	(27) 53·8
	(11) 64·0	—	(24) *61·5	(24) *47·7	(29) 55·1

The values marked * are for fruit which would be judged, by eye, to be slightly over-ripe. Values from heavy ¾-full fruit ripened under tropical conditions were obtained from the data of an earlier publication (Barnell, 1941). The figures in brackets indicate the time in days from cutting.

relatively high in the inferior quality fruit produced by ripening under tropical conditions.

None of the chemical criteria of quality presented are distinctive owing to the range of values within each quality during the stage at which the fruit would be adjudged by the consumer to be eating-ripe. However, for fruit of comparable skin colour it is apparent that that selected from a cargo which has received prolonged cold storage will, most probably, have a higher starch content, lower sugar, more acid, and more glycosidic-glucose (probably derived, in part at least, from tannins) than fruit taken from a cargo which has received a period of cold storage within the known tolerance of the grade of fruit carried.

Another point of difference observed between chilled and unchilled fruit

was the differences in the water losses to the atmosphere from the skin. The water loss of the skin of chilled fruit is mainly accounted for by the uptake of water by the pulp while in unchilled fruit less than half is so accounted for,

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TABLE X

The Pulp/Skin ratio as an Index of Ripeness

</div>

	Days from cutting to 'eating-ripeness'.		
Grade of fruit and storage treatment. (All ripened at 68° F.)	By observation.	Pulp/Skin ratio (interval for change to 2+).	By starch value (falling to < 2%).
Standard ⅔-full 14 days at 53° F.	19–23	22–24	22–24
Standard ⅔-full 20 days at 53° F.	24–29	25–27	27–29
Heavy ⅔-full 7 days at 53° F.	14–18	13–16	16–18
Heavy ⅔-full 14 days at 53° F.	19–23	19–21	21–24

The second column gives the range of time during which the fruits were judged to be eating-ripe by their skin colour alone. Column 3, using the data of Table I, gives the time-interval during which there was an increase in the pulp/skin ratio from not less than 1·70 to values of 2+(1·97 for heavy ⅔-full fruit of the last row), while the last column contains the time-ranges indicated by the fall in starch percentage content of the pulp to relatively low values, i.e. to less than 2 per cent. of taka-diastase hydrolytic products estimated as glucose.

the residue presumably being transpired. So either the chilled fruit has a reduced transpiration rate or there is considerable movement of water from the bunch stem to the pulp of such fruit.

The variability of the pulp/skin ratio or 'coefficient of ripeness' noted by Wolfe (1931) was presumably a result of not using standard grades of fruit, as it has been shown (Wardlaw, Leonard, and Barnell, 1939 *a*), that a steadily increasing value of the ratio is obtained with increasing size of fruit on the plant. Fruits of approximately equal weights show a high degree of uniformity for the values of the ratios, particularly if cut from corresponding positions on the bunches. Leonard (1941) has shown also that this ratio assumes steadily increasing values during ripening at tropical temperatures.

To relate, as closely as possible, the various methods of estimating ripeness in the banana, Table X has been constructed to show the time of attainment of the eating-ripe stage as judged by (1) observation of skin colour of the populations of fruits, (2) the pulp/skin ratio, (3) the percentage starch content (total sugars could be used alternatively), for the two grades of fruit after their respective storage and ripening treatments. There is close agreement, on the whole, between the times indicated for the attainment of eating-ripeness by the three methods, though skin colour tends to give the earliest value, the pulp/skin ratio a middle value, and the chemical index the latest. Palatability depends on the composition of the fruit; therefore it will be, in general,

at its optimum quality a day or so later than suggested by full yellow skin coloration and by a 'coefficient of ripeness' of 2·0.

(d) Correlation of biochemical with physiological studies on the banana.

These biochemical studies, as a whole, link up with work in progress on the respiration, internal gas relationships, and transpiration of the banana fruit. It has already been demonstrated (Wardlaw, Leonard, and Barnell, 1939 *b*), that major chemical changes in the pulp of the banana fruit accompany the profound changes in the internal gas relationships and respiration associated with the attainment of the climacteric by the fruit. The linkage of the information now available on the carbohydrate biochemistry of the banana fruit during development, during various cold storage treatments, and during ripening both under tropical conditions and at controlled temperatures with the data on respiration and gaseous phenomena will contribute towards a foundation for the work in progress on the 'refrigerated-gas-storage' of bananas.

(e) Future lines of biochemical investigation.

Apart from studies in carbohydrate metabolism correlated with respiration and the physiological investigations on the banana fruit, certain other biochemical aspects of the fruit are of considerable fundamental importance. The lines of research which promise to clarify the chemical definition of 'quality', possibly the most urgent problem in the biochemical study of the banana, are: (i) tannin and glycoside metabolism, especially in their relation to chilling of fruits in which there is the possibility of delayed tannin precipitation or oxidation causing astringent flavour and some degree of inhibition of amylase activity, hence slower starch hydrolysis during ripening; (ii) acid metabolism; a close relationship between the titratable acid changes in the pulp and other changes at the climacteric has been observed and also differences in the titratable acid of chilled and normal ripe fruits, so that a careful study of the titratable acid and pH of the expressed juice of the pulp, at least, is desirable; (iii) the considerable changes which take place in the tissue structure of the pulp during ripening suggest that a study of the changes in the composition of cell-wall tissue is necessary; this will require investigations particularly of hemicelluloses, also of cellulose, mucilage, and pectic substances; (iv) during late senescence, at least, it is known that appreciable quantities of alcohol and acetaldehyde are produced in the banana, giving it a 'fermented' odour and taste. For studies of the effects of various methods of storage on the metabolism it is clearly of importance that the relation of alcohol and acetaldehyde[1] contents to quality should be established and that they should be estimated in fruits undergoing various treatments as in the present work on carbohydrates. Alcohol-acetaldehyde metabolism has been

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[1] The interaction of aldehyde and tannin is, probably, of considerable importance in the study of 'quality'.

shown, especially by work on apples, to be of fundamental importance in the interpretation of the respiration phenomena of such senescent organs as ripening fruits; Thomas (1930), Thomas (1931), Fidler (1933 *a*), Fidler (1933 *b*), Thomas and Fidler (1933).

XII. Summary

1. The changes in the amounts of dry matter, starch, sucrose, glucose, fructose, glycosidic-glucose, and in titratable acidity in the pulp and skin of two commercial grades of banana fruit have been determined during various storage treatments.

2. The storage treatments were so arranged that from each of the standard ¾-full and heavy ¾-full grades, fruit was obtained which, when ripened, yielded (*a*) fruit of good eating quality and (*b*) functionally disordered fruit described as 'chilled'.

3. The percentage amount of total dry matter in the pulp showed little change during the cold storage periods at 53° F. but decreased considerably during the ripening period at 68° F. The starch percentage fell somewhat during the first 2 or 3 days from cutting (this includes 1 day at tropical temperatures and then the period of cooling to 53° F.), afterwards increasing slightly in both grades of fruit. Rapid starch hydrolysis occurred during ripening at 68° F.; in those fruit undergoing the prolonged cold storage treatments this increased hydrolysis rate began before transference of the fruit to the ripening room. Total sugars increased in percentage amount slowly through the cold storage periods, the high rate of increase when the fruit was transferred to the ripening room coincided with the high starch hydrolysis rate; in the fruit undergoing prolonged cold storage the accelerated rate of increase of total sugars began before the fruit was transferred to 68° F. The drifts of the percentage amounts of sucrose, glucose, fructose, glycosidic-glucose, and also of titratable acid have been followed and their interrelations and implications discussed.

4. Dry matter in the skins increased slightly but steadily during the cold storage periods, loss of water to the atmosphere and to the pulp presumably occurring. During ripening at 68° F. considerable increase in the percentage occurred, coinciding with the decreasing percentage in the pulp. The starch and total sugar percentage contents followed courses similar to those in the pulp tissue but at relatively low magnitudes and showed a lay period. Analytical data as for the pulp are supplied.

5. The data are also expressed as amounts per single finger in the pulp and in the skin, so that quantitative changes in the amounts of estimated substances during storage and ripening may be observed without the distortion produced by a varying basis such as the fresh weight.

6. The rate of starch hydrolysis in the pulp of ripening fruit has been related to its storage treatment and 'grade'. The rate of hydrolysis is relatively low in 'chilled' fruit and also, most probably, in 'thin' fruit.

7. 'Eating quality' has been discussed in terms of carbohydrate composition of the pulp. A relatively wide range of values for starch and sugar concentrations fall within the limits of the popularly accepted eating-ripe condition. 'Chilling' produces a fruit of poor eating quality and, in general, the pulp of such fruit will have a higher starch content, lower sugar, more acid, and more glycosidic-glucose (possibly derived from tannins) than the pulp of good quality fruit.

8. A value of 2·0 of the pulp/skin ratio or 'coefficient of ripeness' for ripening ¾-full and heavy ¾-full fruits provides an index of ripeness closely agreeing with other criteria such as skin colour and chemical composition of the pulp.

9. The linkage of biochemical investigations with concurrent studies on the respiration and internal gas relationships of the banana fruit is discussed as a basis for future work on storage problems of the banana.

10. The biochemical investigations necessary to clarify further the chemical definition of 'quality' in the banana fruit are outlined, and their potential importance in contributing to knowledge of the fundamental metabolism of the fruit stressed.

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Symbiosis of Leguminous Plants and Nodule Bacteria

II. Further Observations on the Excretion of Nitrogenous Substances from Nodules

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INTRODUCTION

THE phenomenon of excretion of fixed nitrogen from healthy nodules of leguminous plants, to which attention has been drawn by the work of A. I. Virtanen (1938, also earlier papers) in Finland, continues to engage the attention of investigators at several research stations. The problem, still largely unsolved, is to determine the circumstances that govern excretion, and, from the practical standpoint, to decide to what extent excretion is likely to be a feature of legume physiology under field conditions. Shortage of space prevents detailed consideration of several publications dealing with this excretion that have appeared within the last year or two, but they have been reviewed by Wilson (1940) and by Ludwig and Allison (1940). The phenomenon has received considerable attention at the Madison station, and Wilson (1940) has concluded that the absence of excretion in many experiments at that station is due to climatic conditions which preclude the maintenance within the legume of a particular balance between photosynthesis and fixation which is considered to be necessary for excretion to proceed, and which also induce biochemical changes in the nodule that are unfavourable to excretion.

Earlier experiments on excretion carried out in this department gave essentially negative results and have been described in two previous articles (Bond, 1938; Bond and Boyes, 1939). The first article concerned experiments designed to test whether excretion occurred under particular conditions which had been employed previously in experiments dealing with another aspect of legume development. The second dealt with efforts to determine whether excretion could be demonstrated at all at this station. The present paper

describes further efforts in this direction, and some positive results can now be described.

METHODS

The pea (*Pisum sativum* L.) was used in most of these experiments, which, unless otherwise stated, were carried out in this department. In some of the experiments, as a means of detecting excretion, reliance was placed on the up-take by non-leguminous plants growing with nodulated legumes of a propor-tion of any nitrogenous substances excreted by the latter. In some cases this was supplemented by Kjeldahl analyses of the rooting medium, in view of the possibility that excreted products might not be taken up to any great extent by non-legumes (see Virtanen, 1938, p. 42, and Scholz, mentioned by Ludwig and Allison, 1940).

In general methods were similar to those previously described (Bond and Boyes, 1939). The plants were grown in sand-culture in open containers, and in greenhouse experiments the pots, sand, culture solution, and water were autoclaved prior to use, though this step was omitted in the outdoor experi-ments. Seeds were in all cases initially surface-sterilized. Hiltner's nitrogen-free culture solution, as used by Virtanen, with calcium carbonate added, was employed throughout, and the moisture content of the sand was maintained at 12–15 per cent. by weight of dry sand.

TABLE I

Mechanical Analysis of Rooting Media*

Particle size (mm.).	Coarse Quartz. %	Medium Quartz. %	Fine Quartz. %	Coarse Red sand. %	Medium Red sand. %	Super-fine Red sand. %	Finnish sand.† %
>1·00	2	0	0	3	0	0	0
1·00–0·50	18	6	0	4	0	0	0
0·50–0·25	50	43	31‡	22	9	0	9
0·25–0·10	29	49	68	57	71	62§	56
0·10–0·05	1	1·5	1	10	13	28	30
<0·05	0	0·5	0	4	7	10	5

* The analyses were carried out by means of British Standard sieves, with mech-anical agitation. The apertures of these sieves are not in all cases simple fractions of a millimetre, and the figures in the first column are to the nearest second place of decimals. Assistance in these analyses was given by the Mining Dept., Glasgow Royal Technical College.

† From Wilson and Burton (1938).

‡ All less than 0·3 mm.   § All less than 0·15 mm.

In view of the possible influence of the nature (especially the fineness) of the rooting medium on excretion, various grades of sand were employed, and mechanical analyses of these are given in Table I. It will be observed that some of the sands are fairly similar to that used by Virtanen, while others are coarser and one is finer. At the same time it may be noted that more recently

Ludwig and Allison (1940) have given details of a sample of sand received by them from Prof. Virtanen, and on the basis of their figures it appears that sand appreciably coarser than that referred to in Table I has also been employed in the Finnish experiments. The various grades of sand all contained initially a certain proportion of nitrogenous contaminants, but experience showed that for the most part these were non-available to the plants.

As in previous experiments the pea cotyledons were removed after the plants had become well established but before extensive decay of the cotyledons had set in. The arrangement of satisfactory controls for mixed culture experiments presents some difficulty. The method adopted here was to grow the non-legume alone in the control pots, a greater number being grown per pot in order to maintain an approximately equal density of plants. In harvesting the plants the separation of the roots of legumes and non-legumes was effected as completely as possible, and the figures for dry weight and nitrogen content refer to whole plants. 'Dry weight' was ascertained after drying at 95° C. Total nitrogen was determined by the Ranker modification of the salicylic acid-Kjeldahl process. Details of the method of analysing sand are given later, as are other, cultural, details relating to particular experiments.

DATA

Although previous experiments at this station were carried out in natural light, the plants were grown in the greenhouse or in other situations where the light was somewhat restricted. In view of the possible importance of the light factor (Bond and Boyes, 1939), in some of these later experiments the pot cultures were set out on an experimental plot which provided practically unobstructed incidence of light upon the plants. During wet weather the cultures were protected by an overhead screen of glass-substitute supported on a light framework.

Experiment I. The plants of this experiment, data for which are given in Table II, were grown in the outdoor position described above. The figures indicate that the growth of the peas and the fixation of nitrogen[1] were in most cases satisfactory for pot-culture. The growth of the barley plants associated with the Finnish varieties of pea (Torstag and Concordia) in the Coarse Red sand was in no case appreciably different from that of the barley grown alone. Those grown with Gladstone pea were somewhat stronger. These observations are borne out by the figures for nitrogen content of barley. It should be noted that owing to partial destruction by birds, all remaining ears of the barley growing in the Coarse Red sand were discarded at harvest, and the figures for nitrogen content of these barley plants are thus for the vegetative parts only. The pea-potato combination, which in Finland has given very

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[1] In all these experiments an indication of fixation is obtained by making a deduction at the rate of 6 mg. per plant from the figures for nitrogen content of plants, on account of nitrogen derived from the original seeds.

striking results (see below), gave·no evidence of excretion here. Of the cultures grown in the Medium Red sand two gave a negative result, but in the third pot the barley was appreciably stronger and the nitrogen content was some 12 mg. above that of the control. The peas in this pot were, on the other hand, less vigorous.

TABLE II

Data of Expt. I, April 7–July 28, 1939. Glazed pots containing 12·7 kg. of sand. Peas inoculated with HX strain. Barley variety Chevalier

	Pot No.	Dry wt. of peas (gm. per pot).	N-content of peas (mg. per pot).	N-content of non-legumes (mg. per pot).	Plants present.
	1	7·6	258	17·1* ⎫	8 Gladstone pea,
	2	11·9	405	17·7 ⎬	8 barley.
	3	25·9	730	8·2 ⎫	8 Torstag pea,
	4	17·5	493	13·7 ⎬	8 barley.
	5	25·0	652	11·1 ⎫	8 Concordia pea,
Coarse	6	32·2	841	11·4 ⎬	8 barley.
Red sand	7	—	—	(24·8) 11·7‡ ⎫	Controls, 16 barley
	8	—	—	(22·1) ⎬	alone.
	9	Similar to pots 5 and 6		64·1† ⎫	8 Concordia pea,
	10	,, ,, ,,		39·1 ⎬	1 potato (early).
	11	—	—	(130·0) 65·0‡	Control, 2 potato alone.
Medium	12§	8·9	237	14·8 ⎫	8 Concordia pea,
Red	13	8·1	216	16·2 ⎬	12 barley.
sand	14	4·7	125	28·3 ⎭	
	15	—	—	(32·1) 16·1‡	Control, 24 barley alone.

* The figures for N-content of barley in pots 1–8 are exclusive of the ears (see text).
† The figures for N-content of potatoes in pots 9–11 are for tubers only.
‡ For same number of plants as grown with peas.
§ The growth period for pots 12–15 was July 24–Nov. 20, 1939.

These results may be compared with those of parallel Finnish experiments. In one of these (Virtanen, 1938, Table IX) Torstag peas (inoculated with HX strain) and oats (seven plants of each) were grown in pots holding 8 kg. sand over a period of ten weeks. The average final dry weight of the peas from the two pots was 16·3 gm., with a nitrogen content of 574 mg., figures which resemble those for the Torstag peas in Table II. But the nitrogen contents of the oat plants from the Finnish cultures were 126 and 44 mg. respectively (control pot 11 mg.), and while the difference between the two cultures is notable, even in the less successful one the cereals apparently gained some 30 mg. of nitrogen from the peas. In another Finnish experiment (Virtanen, loc. cit., Table XI) eight Concordia peas, growing with one potato plant, attained in twelve weeks a dry weight of 25 gm. and a nitrogen content of 640 mg. The potato plant showed a nitrogen content of 729 mg. (control plant 153 mg.), indicating an uptake of no less than 576 mg. nitrogen from the peas, which evidently effected a much greater total fixation of nitrogen than in the Glasgow experiment.

Experiment II. In this experiment, which was also conducted on the outdoor site, ordinary unglazed earthenware pots were used for some of the cultures. The results, given in Table III, indicate that there was no evidence of benefit

TABLE III

Data of Expt. II, May 1–Aug. 8, 1940. Eight peas inoculated with HX strain and eight barley (var. Spratt Archer) per pot containing 10 kg. sand

		Pot. No.	Dry wt. of peas (gm. per pot).	N-content of peas (mg. per pot).	N-content of barley (mg. per pot).	Pea variety.
Glazed pots	Medium Quartz	1	16·9	448	7·5	} Concordia.
		2	14·9	396	7·8	
		3	19·3	464	10·1	} Maple.
		4	22·0	528	9·5	
		5	12·0	295	8·9	} Gradus.
		6	10·1	250	9·2	
		7	—	—	(21·8) 9·2*	} Controls, 16 barley alone.
		8	—	—	(14·8)	
	Medium Red	9	12·5	295	16·6	} Concordia.
		10	11·0	260	17·2	
		11	12·4	309	7·1	Maple.
		12	—	—	(26·8) 13·4*	Control, 16 barley alone.
Un-glazed pots	Medium Quartz	13	16·7	439	18·8	} Concordia.
		14	15·3	402	27·2	
		15	22·5	550	17·5	} Maple.
		16	23·1	566	30·4	
		17	—	—	(39·3) 19·7*	Control, 16 barley alone.
	Coarse Red	18	16·1	385	21·0	} Concordia.
		19	16·1	385	30·0†	
		20	8·0	209	24·9	} Gladstone.
		21	7·0	183	30·8	
		22	—	—	25·0*†	Control, 16 barley alone.

* For 8 plants.

. † Owing to a mishap no actual figures for N-content of these plants are available. The figures shown are estimates based on notes and photographs made when harvesting.

to the barley plants by association with peas in the glazed pots containing the Medium Quartz sand, the position with the Medium Red sand being much the same. In the unglazed pots the nitrogen content of barley growing with peas in the Medium Quartz sand was in two cases appreciably greater than that of the control plants, the growth being correspondingly better. Though there is slight uncertainty about the figures for nitrogen contents, the growth of the plants in the Coarse Red sand provided less evidence of excretion than in the Medium Quartz. It may be noted that the control barley in both types of sand showed a nitrogen content of about twice that of the corresponding controls in glazed pots (see Table II for controls in Coarse Red sand). The absorption of this extra nitrogen (derived from original sand nitrogen) is

presumably indicative of greater root activity consequent on the use of unglazed pots.

Experiment III. This was carried out during the period May 1–Aug. 10, 1940, by Mr. A. E. W. Boyd (formerly research student in this department) at the Auchincruive (Ayr) country branch of the West of Scotland Agricultural College, by permission of Dr. D. G. O'Brien, Department of Plant Husbandry. Mixed cultures of Concordia pea and Chevalier barley were grown in a cold frame in unglazed pots containing 8 kg. of a sand slightly coarser than the Finnish type. Despite the fact that conditions were more favourable than in the city, the peas made poorer growth than in corresponding experiments in Glasgow, the dry weight of six plants varying from 4·2 to 11·4 gm. The nitrogen content of the control barley was 17 mg. (six plants), and in five out of eight pots of mixed cultures the nitrogen content of the barley plants was essentially similar to the control. The figures for the remaining three pots were 28, 31, and 40 mg. respectively. These increases are rather greater than those noted in the above Glasgow experiments, though they are still small in comparison with typical Finnish results.

Experiment IV. It has been noted (Bond and Boyes, 1939) that the most significant climatic difference between Helsinki and Glasgow relative to the present work is in respect of direct sunlight, of which much more is incident on the former station. In this experiment the plants were grown in the greenhouse and mainly by natural light, but this was supplemented by artificial light from a 1,000-watt lamp (suspended 50 cm. above the plants) on dull days and every evening until 11 p.m. The Medium and Superfine Red sands and also kaolin were used as rooting media in glazed pots containing 3·5 kg. of the medium, while three varieties of pea were included. The experiment, in which barley again formed the detector plant, was conducted from March 21 to June 28, 1939. Growth and fixation by the peas were improved by the extra light, but, though no detailed statement of results is being included, none of the cultures provided evidence of any significant excretion.

Experiment V. Prof. Virtanen has conducted successful experiments during winter months in which the plants were grown essentially under artificial light, and in personal correspondence has provided the author with details of such experiments. It should be possible to secure, at another station, an approximate duplication of the growth conditions prevailing in these particular Finnish experiments, in which case the investigation of the importance of other factors in excretion will be facilitated. Two experiments of this type have been performed in the Glasgow department.

This first one (V) was set up by Mr. A. E. W. Boyd, but was later taken over by the author. The experiment, involving pea-barley cultures, was started during the winter and the plants received the rather meagre natural light that is available in the greenhouse at that season, together with the light from a 1,000-watt gas-filled lamp suspended 30–50 cm. above the uppermost leaves of the plants. The reflector was fitted with a filter through which a current of

water flowed, eliminating any heating effect at leaf-level. The lamp provided an intensity of the order of 1,200 ft.-candles (comparable to the natural light on a moderately cloudy summer day at this station) at the level of the upper-most leaves, and was supplied for eighteen hours daily. The temperature in the greenhouse during the experiment was mostly within the range 50–60° F. Further details are given in Table IV.

TABLE IV

Data of Expt. V, Jan. 17–May 29, 1939. Three Concordia peas and three Chevalier barley per glazed pot containing 1·6 kg. of sand

	Pot No.	Bacterial strain.	Dry wt. of peas (gm. per pot).	N-content of peas (mg. per pot).	N-content of barley (mg. per pot).
Superfine Red sand	1	HX	10·9	233	6·3
	2	HX	11·1	238	7·7
	3	317	4·7	102	7·3
	4	317	7·7	169	8·8
	5	Control, 6 barley alone.			(10·0) 5·0*
Fine Quartz	6	HX	7·2	163	3·8
	7	HX	5·3	120	4·6
	8	317	4·7	111	3·3
	9	317	4·5	107	3·5
	10	Control, 6 barley alone.			(6·2) 3·1*

For 3 plants*

Satisfactory growth and fixation were shown by the peas, especially by the plants of the first two pots, which made as much growth as plants in the out-door experiments and bore well-filled pods at harvest. Though there was some variation from plant to plant, the barley plants associated with peas in the Superfine Red sand tended to be slightly more vigorous than the controls, and their nitrogen content was somewhat greater. A maximum difference of 3·8 mg. does not, however, provide evidence for any extensive excretion. There was little evidence of benefit to the barley in the Fine Quartz sand, either with the Concordia pea or with the Gladstone and Maple varieties in pots not included in the table.

In an experiment details of which were furnished later by Prof. Virtanen, during six weeks' growth under a 1,000-watt lamp (burning continuously, also see footnote on p. 655) two Torstag peas in sterile culture attained a dry weight of 1·7 gm. and showed an excretion of 7·6 mg. nitrogen (average of three cultures) by sand-analysis, representing about 23 per cent. of nitrogen fixed. There is no evidence of excretion on anything like this scale in the Glasgow experiment.

Experiment VI. This was along similar lines to the previous experiment, but this time a 2,000-watt lamp was maintained at a height of 30 cm. above the uppermost leaves of the plants, giving there an intensity of 2,400 ft.-candles, and the lamp was burned continuously. The experiment was con-ducted in a dark room provided with forced ventilation, which, in conjunction with the water-filter, allowed of the maintenance of a temperature of 50–60° F.

in the room during most of the experimental period. The number of peas was increased to four with the object of securing a greater excretion per pot, while eight barley plants were grown in each pot in order to give efficient uptake of any excreted substances.

TABLE V

Data of Expt. VI, Nov. 8, 1939–Jan. 27, 1940. Four Concordia peas (inoc. HX) and eight Spratt Archer barley per glazed pot containing 1·8 kg. of sand. Original N content of Superfine Red sand = 102 mg. and of Fine Quartz = 9·5 mg. per pot

	Pot No.	Dry wt. of peas (gm. per pot).	N-content of peas (mg. per pot).	N-content of barley (mg. per pot).	N-content of sand (mg. per pot).
Superfine Red sand					
First harvest	1	6·1	125	7·5	97
	2	5·8	119	7·9	98
	3	4·0	81	7·8	—
Second harvest	4	9·2	243	10·0	102
	5	8·4	180	7·4	98
	6	7·2	166	7·8	—
	7	Control, 12 barley alone.		(14·8) 9·8*	97
Fine Quartz sand					
First harvest	8	2·6	68	5·2	16·3
	9	2·1	56	5·6	15·4
	10	2·0	51	5·9	—
Second harvest	11	2·9	71	5·6	17·7
	12	2·4	59	5·4	16·9
	13	2·2	54	5·5	—
	14	Control, 12 barley alone.		(7·6) 5·1*	12·4

* For 8 plants.

It will be noted from Table V that in general the growth and fixation of nitrogen by the peas were below those of the previous experiment, perhaps because of closer planting and continuous illumination. The growth and nitrogen content of the barley plants provided no evidence of excretion in either type of sand. Though no control pots were included at the first harvest (at eight weeks), the appearance of the barley grown with peas was essentially identical with that of barley growing alone. The same was true at the second harvest (twelve weeks), and the figures for this stage show that there was no significant superiority of nitrogen content.

Sand-analyses were effected in connexion with some of the pots. The method adopted (Bond, 1938) involved the analysis by the Kjeldahl process of four samples (each of 200 gm. in the case of the Fine Quartz and of 150 gm. in that of the Superfine Red) of the dried sand from each pot, root fragments being removed by sieving. Further tests using this method gave practically 100 per cent. recovery of asparagine nitrogen added to 200 gm. samples of the quartz sand, and only slightly lower recovery with the finer red sand.

Table V indicates that the Superfine Red sand (which consisted of the finest screenings of the Coarse Red sand) contained at the start of the experiment an appreciable amount of nitrogenous impurity, consisting presumably of organic debris, and representing a little more than 100 mg. nitrogen per pot, despite the fact that before use all this sand was ignited at a temperature of 400–500° C. However, the low nitrogen content of the control barley shows that this original nitrogen was essentially unavailable to those plants, and presumably the same holds for the peas. The figures show that the nitrogen content of the sand remained essentially at the original level in all the pots containing the Superfine Red sand, so that no evidence of excretion is forthcoming from sand-analysis.

The results for the Fine Quartz sand show that the nitrogen content of sand in which peas and barley had grown increased by several milligrammes over the original figure (which was much lower than that for the red sand), some increase being shown by the control pot as well. A similar result was obtained in earlier experiments with quartz sand, and the origin of the small increases has been discussed (Bond and Boyes, 1939). It is clear that at the most they provide evidence of only trifling excretion.

In an experiment carried out by Prof. Virtanen, two peas (var. Torstag, similar to Concordia) grown alone in the sterile system in Woulff's bottles under a 2,000-watt lamp[1] attained a dry weight of 4·3 gm. (average of three bottles) over a period of six weeks, with a nitrogen content of 74 mg., and showed an average excretion of 21 mg. nitrogen per bottle by sand-analysis. This again represents about 23 per cent. of the nitrogen fixed. In the author's experiment, after eight weeks' growth the best pot of peas showed a dry weight of 6·1 gm. (four plants) and a nitrogen content of 125 mg. Evidently, in addition to showing no excretion, these peas did not make quite such vigorous growth as those in the Finnish experiment, probably because of the closer planting.

Experiment VII. This experiment, to which brief reference has been made already by Wilson (1940, p. 158), was set up by the present author while he was working in the Dept. of Agricultural Bacteriology of the University of Wisconsin, and he wishes to express appreciation of the many courtesies extended to him during the period. The plants were grown in the glasshouses of the Dept. of Horticulture, with the co-operation of Prof. R. H. Roberts. Previous experiments (Wilson, loc. cit.) had provided evidence that at the Madison station excretion tends to occur from peas grown during the winter and spring months, when it is possible to maintain a moderate temperature in the glasshouse, favourable to the growth of peas, while the light is adequate to support good growth, the average total hours of sunshine per

[1] In this and the previous Finnish experiment the lamp was kept only 10–20 cm. above the uppermost leaves, the light intensities at that level being about twice those mentioned for the author's experiments. The light values for lower leaves would depend on the spacing of the plants.

month being as follows: December, 102; January, 127; February, 145. The present experiment was set up in order to confirm and investigate further the occurrence of excretion under these conditions, and was actually continued for some time after the author's departure, the final data being recorded by the research staff of the Madison department.

TABLE VI

Data of Expt. VII, Nov. 5, 1938–Feb. 24, 1939. Glazed pots containing 2·5 kg. of sand. Peas inoculated with Strain 317

	Pot No.	N-content of peas (mg. per pot).	N-content of barley* (mg. per pot).	N-content of sand (mg. per pot).	Pea variety.
Quartz sand	1	73 (4)†	12·6	26	Gladstone
	2	88 (3)	10·5	25	
	3	79 (4)	8·8	24	Canada Field
	4	64 (5)	13·8	26	
	5	Control, 16 barley alone.	9·6‡	20	
Pit sand	6	73 (4)	17·7	30	Gladstone
	7	75 (4)	20·1	25	
	8	77 (3)	17·5	23	
	9	76 (4)	23·3	24	Canada Field
	10	81 (5)	12·3	27	
	11	108 (5)	16·5	24	
	12	Control, 16 barley alone.	9·8‡	22	

* 10 plants per pot, variety Wisconsin Beardless.
† Number of plants present.
‡ For 10 plants.

A section of the data is presented in Table VI. The 'pit sand' was rather similar to the Coarse Red sand of Table I, while the 'quartz sand' was similar to the Coarse Quartz of that table (see Wilson and Burton, 1938, for full description). The cultures included in Table VI were grown in a glasshouse adjusted to a minimum temperature of 55° F., though on sunny days this was appreciably exceeded. The natural photo-period was extended for several hours each day by low-intensity artificial light. It will be noted that the nitrogen content of barley associated with peas in the finer (pit) sand was in all cases greater than that of the control, the growth being correspondingly stronger. The gains in the quartz sand were smaller. With both types of rooting medium the final nitrogen content of the sand tended to be higher in the pots containing peas than in the control. In a second group of cultures (not shown in the table) grown in a house adjusted to a minimum of 65° F., the barley benefited less by association with peas.

Besides the above experiment, another one was conducted at Madison by the author during the autumn months, when much higher temperatures and light intensity prevail in the glasshouse. No evidence of excretion was provided by mixed cultures of barley with pea, Soya bean, and broad bean (*Vicia Faba* L.) in open pots.

DISCUSSION

The results of Experiments V and VI will be considered first, since it was probably in them that conditions approached most nearly to those prevailing in corresponding Finnish experiments. Thus the lighting and temperature must have been very similar, while the Concordia pea inoculated with the HX strain of bacillus has been much used in Virtanen's experiments. Though the Fine Quartz sand was coarser than the Finnish sand, the Superfine Red was actually finer and should have been favourable to excretion. Despite these considerations, while there was possibly slight evidence of excretion in Expt. V, it was not confirmed in the following experiment.

In the Finnish experiments with artificial light, of which details are given above, the closed, 'sterile' system of culture was employed, while in the author's experiments the cultures were in open pots, only initially sterile. This does not seem likely to have been responsible for the different result, since Virtanen has observed excretion in both types of culture and finds that it tends to be greater in the non-sterile arrangement. Virtanen and Torniainen (1940) have concluded that the aeration of the rooting medium plays an important part, and according to their view, for a satisfactory demonstration of excretion in open, mixed cultures there must be liberal aeration of the medium, such as is provided by unglazed pots of as high porosity as possible. In these Glasgow experiments glazed pots were used. There seems, however, to be a discrepancy between the conclusion reached by Virtanen and Torniainen and the very successful use in many Finnish experiments of glass containers, such as suction flasks or Woulff's bottles, with their narrow apertures plugged with wool. Aeration of course depends not only on the type of container, but also, among other factors, on the density of planting. Though the amount of sand used in the Finnish artificial-light experiments is not indicated, reference may be made to experiments of Virtanen and von Hausen (1935, Table I) which illustrate the effect of density of planting upon excretion. In the densest planting four Torstag peas were grown in a suction flask containing 1·3 kg. of sand, the final excretion being of the order of 20 per cent. of the nitrogen fixed. With four plants in 2·7 kg. of sand the excretion increased to 33 per cent. In Expt. V of the Glasgow series three peas and three barley plants grew in 1·6 kg. of sand; bearing in mind the smaller growth of the barley, the density of planting here was probably much the same as in the first of the Finnish experiments, but the aeration seems likely to have been better because of the open container. The reason for the comparative failure of this experiment remains obscure, unless it is connected with differences in photo-period or in the position of the plants in relation to the source of light. In Expt. VI, where four peas and eight barley were grown in 1·8 kg. of sand, the density of planting was greater than in the most densely planted Finnish experiment, and though the open container would provide a measure of compensation, it is possible that the aeration of the rooting medium was below the minimum for excretion.

Attention may now be directed to the results of Expts. I, II, and III, conducted under outdoor conditions which were normal for the district so far as light intensity, photo-period, and temperature are concerned. The majority of the cultures in these experiments provided no evidence of excretion. Thus out of the thirty-six mixed cultures involved, only in nine (possibly ten) cases did the nitrogen content of the non-legumes associated with eight nodulated peas exceed that of the controls by more than 5 mg., and even in these cultures the increases were much below those characterizing the more successful experiments of Virtanen.

Assuming that this absence of benefit to the associated plants is actually indicative of the lack of excretion, and not of a failure of the plants to utilize excreted products, the problem is to decide whether the high proportion of negative results obtained in these outdoor experiments, and the rather meagre nature of such positive results as were observed, signifies that climatic factors in this area are less favourable to excretion than at the Finnish station, or whether the excretion was hindered by some other feature of the experimental arrangement. This is an important point, for if the former conclusion is reached, then the implication is that excretion plays little part in the nitrogen economy of a mixed crop such as 'mashlum' (broad bean and oats) which is widely grown in this country, or the various grass-clover mixtures. It has been pointed out (p. 652) that the most significant climatic difference between the Helsinki and Glasgow stations during the summer months is probably in respect of the amount of direct sunlight, and hence of average light intensity, and the more rapid growth which is frequently indicated by the data of Finnish experiments is doubtless due to the better light. The climatic differences between the two stations are, however, nothing like so marked as those between Helsinki and Madison, for which station Wilson, as stated already, has concluded that conditions are during most of the year unfavourable to excretion, and it is clear that other possibilities should be examined thoroughly before a final conclusion is drawn.

Non-climatic factors which might be responsible include again the type of rooting medium and of container, and density of planting. With regard to the first, Table I shows that, of the various grades of sand used in these outdoor experiments, the Medium Quartz is distinctly, and the Coarse Red slightly coarser than the Finnish sand, the Medium Red being quite close to the latter. The sand used in Expt. III was also only slightly coarser than the Finnish grade. With these facts in mind, together with the probability that coarser sands have also been used at Helsinki, it seems unlikely that the general tendency towards negative results was due to the use of too coarse media. Actually these experiments do not provide evidence of any effect of the fineness of the sand within the range employed, since such small positive results as were observed occurred in all four grades of sand.

In rather more than half of the outdoor cultures, large glazed pots provided with a basal tubulure were used. Glazed pots have several advantages over

the unglazed type for general experimental purposes, and there seemed no objection to their use in the investigation of excretion, especially in view of the employment of glass containers in Finnish experiments. Recently, as noted above, Virtanen and Torniainen have urged the use of unglazed pots for open cultures, though it should be noted that some excretion was observed in glazed pots by Nowotnówna (1937) and also in Expt. VII above. On the other hand, it is true that in the Glasgow outdoor experiments a rather higher pro-portion of relatively positive results was obtained in unglazed pots than in the glazed type, though there is difficulty in accepting this as a result of the im-proved aeration, for in both types of containers the plants were much less densely sown than in some Finnish experiments in closed glass containers (p. 657), so that the aeration even in the glazed pots seems likely to have been superior to that in the latter experiments. The matter requires further investigation.

The fact that positive results were obtained in the winter experiment at Madison calls for comment, although again the excretion was slight compared with that in many Finnish experiments. That greater success attended this experiment than those of the Glasgow series appears most likely to have been due to climatic factors, for the general arrangement of the experiments was identical. The particular combination of moderate temperature and fairly good light under which the Madison experiment was conducted does not obtain here for any lengthy spell during the year. Apparently this is one type of climate that favours excretion. The fact that negative results were obtained in the autumn experiment at Madison shows clearly that climatic factors may play a decisive part in determining excretion. The winter experiment con-firmed this in respect of temperature, and also indicated that relatively coarse rooting media are unsuitable for excretion.

In conclusion, it appears that for appreciable excretion to occur a particular combination of circumstances is necessary. It is not sufficient merely that conditions should be such as produce good growth of the nodulated legume. Virtanen has evidently secured the necessary conditions without special effort at the Helsinki station, but this has not been the case with other investigators. One is bound to question the significance of a phenomenon which seems to occur relatively infrequently under many experimental conditions, unless it is that the special precautions necessary to secure more pronounced excretion actually make conditions more natural and akin to those prevailing in the field.

SUMMARY

Experiments were carried out in the Glasgow area and also at Madison, Wis., U.S.A., designed to detect excretion of nitrogenous substances from nodules of legumes (mostly peas) growing in sand-culture. Both the asso-ciated-growth and sand-analysis methods were employed.

In the Glasgow series, which included experiments under natural and arti-ficial light, many negative results were obtained, but in a proportion of the

cultures there was evidence of some excretion, though this was slight in comparison with that observed in many of Virtanen's experiments in Finland. Some positive results were also obtained in the Madison tests.

In the course of the experiments evidence was obtained that climatic factors, fineness of rooting medium, and nature of the containers may affect the amount of excretion. It cannot yet be decided finally whether the general tendency towards negative results in experiments carried out in the Glasgow area under natural conditions in respect of light and temperature is due to climatic conditions being unsuitable for excretion, or to some other feature of the experimental arrangement.

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Studies on the Nitrogen Metabolism of Plants

IV. On the Changing Nature of the Relation between Proteins and Amino-Acids

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With five Figures in the Text

INTRODUCTION

THE work described in previous papers of this series (Petrie and Wood, 1938*a* and *b*) has led to the conception of a steady state in the leaves of plants at which there is a certain relation between the amount, P, of protein, and the amount, A, of amino-acids. The evidence suggested that parameters in the relation are the pH of the expressed sap, the water content of the leaves, and possibly other quantities. On theoretical grounds (Petrie and Wood, 1938*b*) it was also expected that the form of the P-A relation would depend on the relative proportions of the various amino-acids. That a steady state of this type would ever be maintained in the plant was not expected: but it was regarded as possible that conditions could be such as to permit the establishment of a drifting steady state; here the determinants of the steady state change so slowly that there results a succession of states, each of which is inappreciably removed from a steady one. The conception of reversible, drifting steady states was of value when interpreting the considerable changes in the variables produced in the experiments discussed in the previous papers of this series. It was not shown that such states were attained, but it was held that they were sufficiently closely approached for the data to reveal certain properties of the steady-state P-A relation.

These properties were deduced for a relation observed only for a brief period of time, in which it was thought probable that marked ontogenetic changes in the tissue did not occur; it is proposed now to take up the problem of possible changes in the P-A relation during longer periods of time, particularly when the tissue is passing through the stages of maturity and senescence.

The investigation of this problem has already been commenced by Walkley (1940), who observed the drifts of certain attributes of the fully expanded fourth leaf of barley. It was found that, while the absolute water and total soluble-nitrogen contents remained constant over a long period, P continuously fell. For the fall in protein content of the ageing leaf two alternative interpretations were suggested. According to the first of these only one P-A relation may be concerned and, on account of the existence of external sinks, A continuously falls; a fall in P will result, keeping pace more or less closely with the requirements of the drifting steady state. If this interpretation applied in the experiment concerned, the value of A must so have drifted that, when added to that of the corresponding amount of the remaining soluble-nitrogen compounds, a constant quantity was always obtained. According to the second interpretation, the P-A relation may change continuously so that, for a given A, the steady-state value of P becomes progressively smaller; this was defined as an intrinsic senescent change in the leaf, the effect of which may be superimposed on that of the external sinks.

There was nothing in the data of Walkley's experiment that definitely precluded the first interpretation; it was decided, however, to investigate further the relation between the senescent drifts in proteins and amino-acids. As in the former experiment, this was also studied when extra nitrogen was added after the normal decline in protein content was in progress. It was particularly desired to discover whether under these circumstances there is a restoration in terms of the same P-A relation, or whether a different relation is attained; or finally whether there is possibly a continuous condensation of amino-acids to protein until A is reduced to its original low value,[1] thus invalidating the previous steady-state conception. The results of this further investigation are described in the following pages.

EXPERIMENTAL PROCEDURE

Experiment V. The previous experiment on the fourth leaf of barley (Walkley, 1940) will, in the present context, be referred to for convenience as experiment V. In this experiment the same amount of ammonium sulphate was applied at three different times, but only protein and total soluble nitrogen were determined. It was considered of interest now to supplement the results by a determination of the total amino-acids in the stored, dried material. For this purpose 1 gm. of dried material was extracted with three successive quantities of 10 ml. of an acetate buffer of pH 4·0 at 80° C. for a total period of twenty minutes. No correction in the values was made for the presence of glutamine.

Experiment VI. Cape barley (*Hordeum vulgare* L.) was set to germinate on February 26, 1940 (day 0), and was subsequently planted out in pots arranged in two blocks in the glass-house. The pots contained 4 kg. of a mixture of

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[1] Such a type of steady state is referred to by Borsook and Huffman (1938).

TABLE I. *Results of Experiment VI.*

The values for length × breadth are in sq. cm. per leaf, those for the remaining quantities in mgm. per leaf; asparagine and glutamine are expressed by half the nitrogen of the molecule. Each value is a mean of two block values.

Analyses of variance were performed on certain of the data between harvests 3 and 7 inclusive; the chief results of these are presented with the corresponding probability levels ($\mathscr{P}$): The block effect was significant ($\mathscr{P} < 0.05$) in all cases except the total amino-acids.

				Harvest				
Treatment	1	2	3	4	5	6	7	8
DRY WEIGHT								
Control	61·5	61·6	55·4	59·6	60·7	60·4	64·0	—
I	—	—	59.7	61·4	60·4	62·3	67·8	69·1
II	—	—	61·4	62·0	65·9	64·6	68·8	70·8

Time and treatment significant ($\mathscr{P} < 0.01$); interaction not significant ($\mathscr{P} > 0.05$).

LENGTH × BREADTH								
Control	29·8	28·5	27·7	29·2	29·1	28·3	27·0	—
I	—	—	29·3	29·0	28·0	28·7	28·2	28·4
II	—	—	29·4	29·0	29·0	28·7	27·4	28·0

Time, treatment, and interaction not significant ($\mathscr{P} > 0.05$).

WATER								
Control	34·6	34·6	33·1	34·5	34·7	34·8	33·8	—
I	—	—	35·0	34·0	33·3	35·0	34·7	33·6
II	—	—	34·5	34·0	34·7	34·7	32·8	33·9

Time, treatment, and interaction not significant ($\mathscr{P} > 0.05$).

PROTEIN NITROGEN								
Control	2·46	2·21	2·08	1·98	1·62	1·41	1·23	—
I	—	—	2·50	2·63	2·37	1·96	1·56	1·43
II	—	—	2·48	2·67	2·96	2·60	1·87	1·76

Time, treatment, and interaction significant ($\mathscr{P} < 0.01$).
Standard error of mean of two block values = ±0·050.

TOTAL SOLUBLE NITROGEN								
Control	0·232	0·182	0·160	0·153	0·134	0·138	0·138	—
I	—	—	0·372	0·318	0·190	0·184	0·150	0·149
II	—	—	0·522	0·398	0·326	0·258	0·164	0·171

TOTAL AMINO-NITROGEN								
Control	0·156	0·105	0·082	0·078	0·068	0·058	0·057	—
I	—	—	0·212	0·186	0·108	0·086	0·056	0·057
II	—	—	0·358	0·222	0·184	0·134	0·068	0·060

Time, treatment, and interaction significant ($\mathscr{P} < 0.01$).
Standard error of mean of two block values = ±0·0135.

RESIDUAL AMINO-NITROGEN								
Control	0·138	0·086	0·067	0·064	0·050	0·041	0·038	—
I	—	—	0·144	0·140	0·082	0·068	0·036	0·036
II	—	—	0·232	0·154	0·128	0 093	0·046	0·039

ASPARAGINE AMIDE-NITROGEN								
Control	0·010	0·014	0·011	0·011	0·013	0·014	0·015	—
I	—	—	0·033	0·028	0·014	0·015	0·015	0·016
II	—	—	0·076	0·038	0·036	0·025	0·016	0·016

GLUTAMINE AMIDE-NITROGEN								
Control	0·008	0·005	0·004	0·004	0·004	0·004	0·005	—
I	—	—	0·035	0·018	0·010	0·004	0·005	0·005
II	—	—	0·055	0·030	0·020	0·006	0·005	0·006

AMMONIA NITROGEN								
Control	0·008	0·009	0·010	0·011	0·010	0·011	0·010	—
I	—	—	0·014	0·016	0·011	0·012	0·011	0·010
II	—	—	0·019	0·018	0·015	0·014	0·010	0·011

two parts Waite Institute loam and one part sand, and the water content of the mixture was maintained constant. After thinning on days 7 and 8 each pot contained 12 plants. On day 10, 0·3 gm. Ca $(H_2PO_4)_2.H_2O$, and on day 17, 0·2 gm. $(NH_4)_2SO_4$, was supplied to each pot.

Preliminary daily measurements of the length of the fifth leaf of a selected number of plants indicated that no further growth took place after day 35. On day 34 the main shoot above the ligule of the fifth leaf and all the tillers on every plant were removed. Thereafter the plants were kept continually pruned of all new growth in order to divert the nutrient stream as far as possible to the fifth leaf.

On days 36 and 39 two preliminary harvests (1 and 2) were taken, and analyses showed that the protein content of the leaf was already decreasing with time. On day 40 a random selection of pots in each block was given 1 gm. $(NH_4)_2SO_4$, which represented treatment I; a further random selection in each block received treatment II, namely 2 gm. $(NH_4)_2SO_4$. The remaining pots constituted the controls.

On the afternoon prior to each harvest six pots of each set from each block were chosen at random and placed in cabinets maintaining a constant temperature of 24° C., a light intensity of 800 metre-candles, and approximately constant humidity. On the day of harvest the fifth leaf on each plant was severed at the ligule and the length and breadth were measured, the product of these being taken as an index of the area as in experiment V. Tracings of leaves showed that the actual area was approximately 70 per cent. of the product of the linear dimensions. The seventy-two leaves were then bulked and chaffed; a sub-sample of 10 gm. was weighed out and used for protein and soluble-nitrogen estimations, and the remainder was weighed and dried in a current of air at 80° C. for exactly one hour and then re-weighed. The usual overnight drying period was curtailed and controlled in this way so that amide determinations could be carried out later on the dry samples according to the method of Vickery *et al.* (1935). The material was quite brittle and ground easily to a fine powder.

One fresh and one dry sample were in this way taken from each block for every value recorded. The 10 gm. fresh sample was ground with purified sand and tungstic acid and filtered; the nitrogen content of the residue was taken as a measure of protein nitrogen. The total soluble nitrogen, ammonia nitrogen and amino-nitrogen were estimated in the filtrate as in previous work (Petrie and Wood, 1938a), except that for the ammonia nitrogen the extract was brought to pH 9·5 with a borax buffer as recommended by Pucher *et al.* (1935). Asparagine- and glutamine-amide nitrogen were determined on the dry material according to the method of Vickery *et al.* (1935), and the amino-acid values obtained from the extract of the fresh material were corrected for the reaction of glutamine-amide nitrogen in the van Slyke apparatus. This correction was negligible in the low values of the controls and the later harvests of the treated plants, but at harvest three of the latter plants was of the order

of 11–12 per cent. of the van Slyke value. The possibility is not excluded that the fraction termed glutamine may have included other similarly unstable amides.

DISCUSSION

General Survey of Results

The dry weight of the leaf in experiment VI was shown by analysis of variance (Table I) to have increased significantly with both time and treatment, as also happened in Experiment V; the increases were regarded as due to accumulation of reserve material and thickening of cell walls (cf. Watson and Petrie, 1940). The area and absolute water content, on the other hand, remained approximately constant: analyses of variance of the data of harvests 3–7 inclusive showed no significant effect of time or treatment, and the values at harvests 1, 2, and 8 did not differ appreciably from those at harvests 3–7. It was therefore concluded that the changes observed in other attributes characterized a mature or post-mature organ in which cell division and cell extension had ceased; in other words, changes in the extensive properties of the intracellular system were minimal, which increases the validity of comparing, at different points in time, values of attributes expressed in absolute terms.[1] If such changes were inappreciable it is possible that the relations revealed between absolute amounts of proteins and amino-acids hold also between their concentrations. Since also water content has been shown to be a major determinant of the relation between proteins and amino-acids in the total leaves of plants (Petrie and Wood, 1938a), its constancy in the present case was expected to facilitate the revelation of effects of determinants possibly obscured in previous work.

The contents of all nitrogen fractions determined, which were either declining or relatively constant at the commencement of observations, rapidly rose with ammonium sulphate treatment (Figs. 1 and 2). The values obtained with treatment II were generally higher than those with treatment I throughout the experiment. Falls subsequently commenced in all fractions, but net protein synthesis continued for some time after the falls in the contents of soluble-nitrogen fractions were in progress. By harvest 8 the contents of the soluble-nitrogen fractions of the treated plants had fallen to the values obtained for the controls at harvest 7, which by then had become almost constant; the corresponding protein contents, however, were higher.

Fig. 3 illustrates the way in which protein content changed with change in total amino-nitrogen content. It is seen that the points for the three sets of

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[1] Unfortunately, changes in extensive properties of the system concerned in protein synthesis cannot be wholly excluded. Although water content may have remained constant, accumulation of reserves may have increased the amount of adsorbed water, or the amount transferred from the protoplast to the cell walls. Treatment may also have caused change in distribution of water between cytoplasm and vacuole. It is interesting, nevertheless, to note that large losses of protein from the leaf can occur without change in water content.
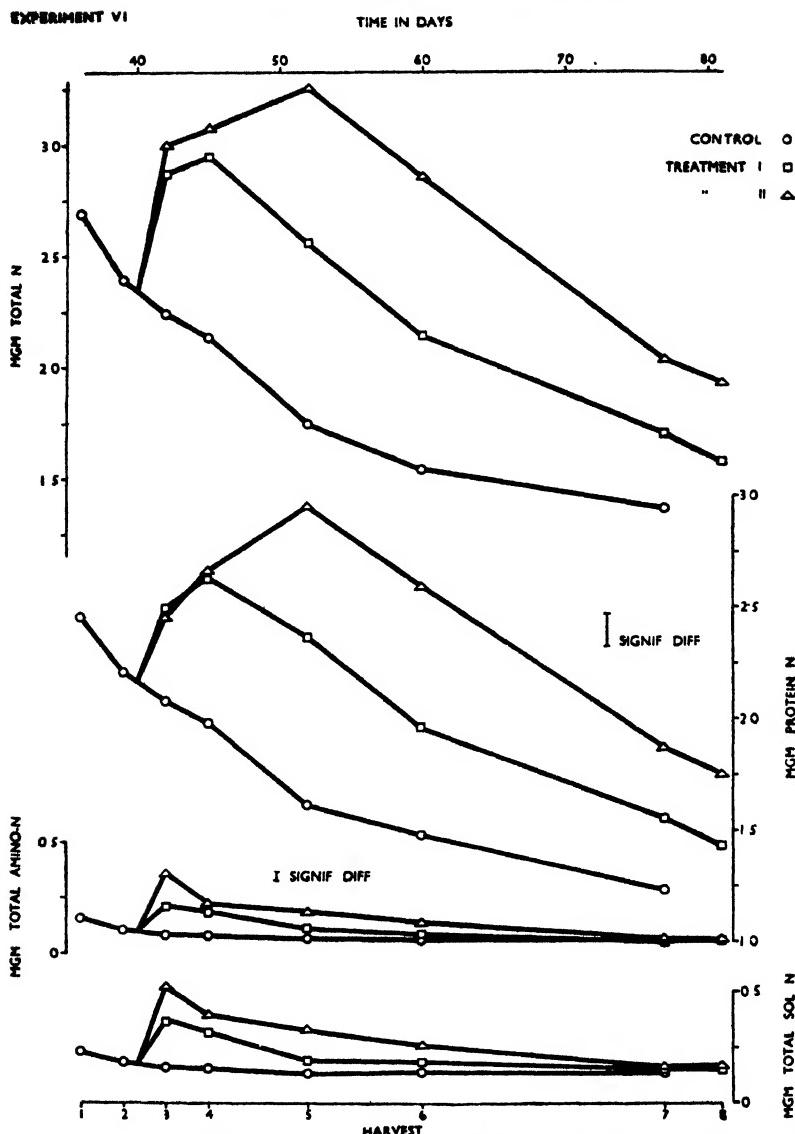
FIG. I. Drifts with time in absolute amounts of various nitrogen fractions in the fifth leaf of barley. Each point represents the mean of two block values. The significant differences which have been calculated for certain of the fractions in this figure and in Fig. 2 represent $t\sqrt{2} \times$ the standard error of the mean of two observations ($n = 30$; $\mathscr{P} = 0.05$); they are applicable to the comparison of individual points lying between harvests 3 and 7 inclusive. The upper time scale is calibrated in days from sowing.

plants fall approximately on three curves, concave to the amino-nitrogen axis, converging in the central region and diverging at the extremities. The curves resemble those obtained by Petrie and Wood (1938*a* and *b*), except that the values obtained during the phase of net protein synthesis (viz. treatment I,

harvest 3, and treatment II, harvests 3 and 4) lie beyond the maxima. The quadratic regression of protein content on amino-nitrogen content for the phase of net hydrolysis was calculated for each set of data, the coefficients


EXPERIMENT VI
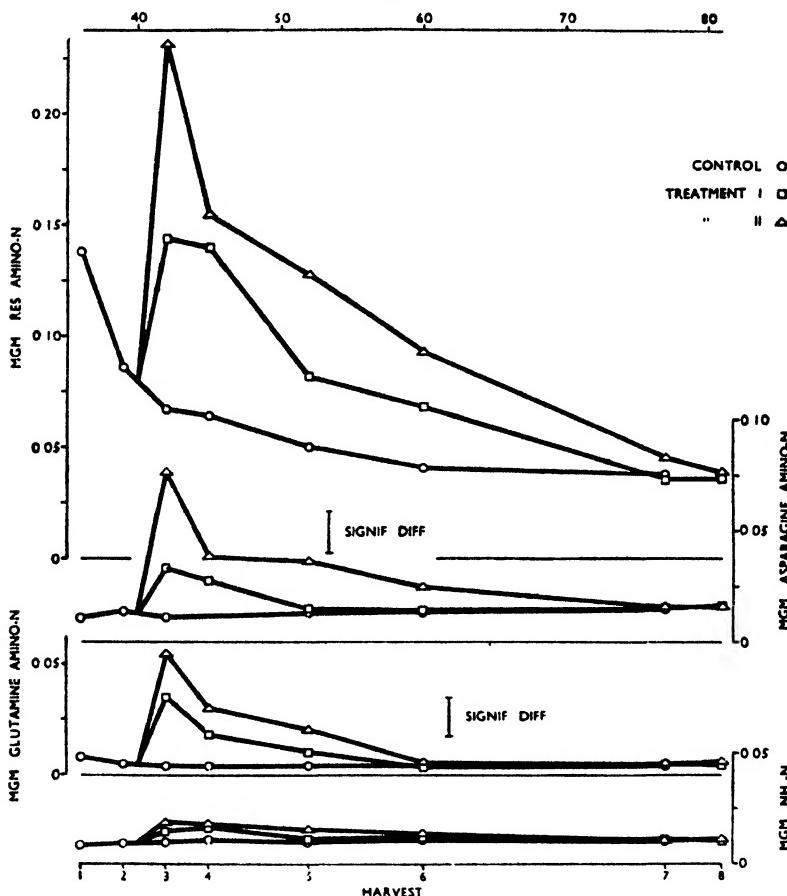
FIG. 2. Drifts with time in absolute amounts of soluble-nitrogen fractions in the fifth leaf of barley. Each point represents the mean of two block values. Residual amino-nitrogen represents the total free amino-nitrogen less the amino-nitrogen of the amides. It will be observed that the values given for the amides are half their total nitrogen content.

being given in Table II and the corresponding curves being plotted in Fig. 3. Although the true relation appears to be steeper near the origin in the case of the controls and treatment I than that expressed by the regression functions, the equations account for a very high percentage of the variance of P. The points for the synthetic phase of treatments I and II on the contrary are not comprised in the same functions. The value of these curves is that they are shown in Table II to have significant differences in form. It is, however,
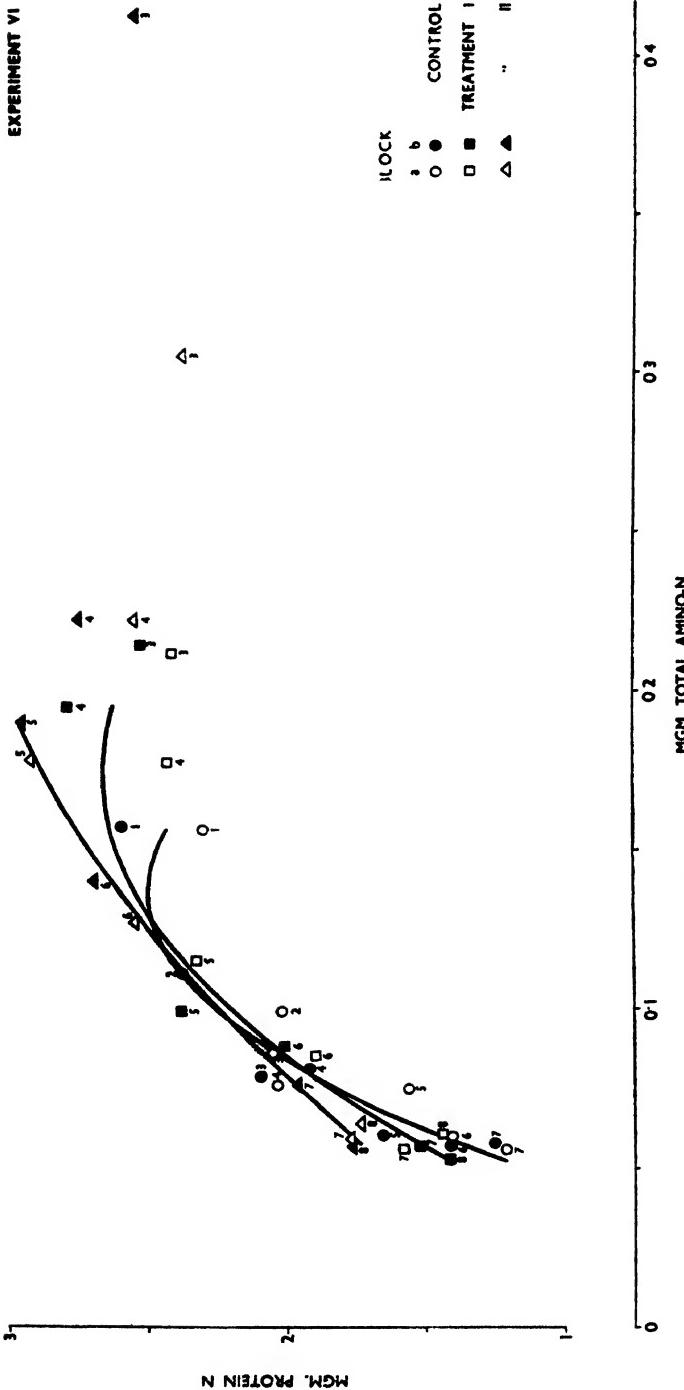
FIG. 3. Absolute protein-nitrogen content plotted against absolute total amino-nitrogen content in the fifth leaf of barley. In this graph the individual block values are given, and the harvest number is indicated for each point. The curves are those of the three regression equations, details of which are given in Table II.

impossible to judge whether their congruity in the intermediate range of amino-nitrogen values has any physiological significance: the treatment effect, which is significant at the extremities, may conceivably have existed over the whole of the phase of hydrolysis, although the present data are insufficiently good to demonstrate this.

TABLE II

Regression Equations.

The data of each series were fitted to the regression equation

$$P = a + b_1 A_T - b_2 A_T^2,$$

where P and A_T are the absolute amounts of protein and total amino-nitrogen per leaf respectively. The variance due to the blocks in the glass-house was removed in the course of the fitting. The results are given below, where V is the percentage of the variance of P ascribable to the average effect of the independent variables, and is derived as in previous papers.

	Harvest range	a	b_1	b_2	V
Control	1–7	−0·90	49·83	181·82	91·85
Treatment I	4–8	+0·09	29·75	85·44	93·55
Treatment II	5–8	+0·82	17·74	33·57	99·75

Both coefficients of the control are significantly greater than those of both treatment I ($\mathscr{P} < 0·05$) and treatment II ($\mathscr{P} < 0·01$); the coefficients of treatments I and II are not significantly different.

At $A = 0·058$, $P_I^{(calc.)}$ and $P_{II}^{(calc.)}$ differ significantly ($\mathscr{P} < 0·05$), while $P_{control}^{(calc.)}$ is not significantly different from both $P_I^{(calc.)}$ and $P_{II}^{(calc.)}$; at $A = 0·156$, significant differences occur between $P_{control}^{(calc.)}$ and $P_{II}^{(calc.)}$ ($\mathscr{P} < 0·01$), but not between $P_{control}^{(calc.)}$ and $P_I^{(calc.)}$, or $P_I^{(calc.)}$ and $P_{II}^{(calc.)}$.

In experiment V the same treatment was applied to different sets of plants on different occasions, but the drift in the treated plants was followed only for a short period of time, only in fact while the protein nitrogen was still increasing. Fig. 4 shows that in the controls there was a continuous fall in the amino-nitrogen content, although the total soluble-nitrogen content remained constant; the loss in amino-nitrogen must have been approximately counter-balanced by increase in other fractions. The relation between proteins and amino-acids during the phase of hydrolysis (Fig. 4) resembles that of experiment VI. Furthermore, the lower P had fallen prior to the application of treatment, the greater was the apparent lag in synthesis: in neither experiment did the system retrace the path of the P-A relation of the hydrolytic phase.

In Fig. 5 are shown the changing proportions of the amino-acids in experiment VI. The normal drift was in the direction of increasing proportions of asparagine as the leaf aged. On the other hand, the application of ammonium sulphate caused a temporary increase in the proportion of glutamine.

The Approach to the Protein Maximum.

The observations suggest that the curve relating P and A during the hydrolytic phase of the experiment reaches its highest point at the highest A value (Fig. 3). The regression functions, it is true, in the cases of the control and

treatment I plants display maxima at slightly lower A values, but the falls beyond the maxima are inappreciable within the range of the data. There is, however, a maximum in the curve if the data for the phase of net synthesis are included.[1] Two alternative explanations may be suggested for this property. In the first place there may have been a lag in the approach to a steady state, which was perhaps attained at the protein maximum; in other words, the amino-acids may have accumulated more rapidly than they were utilized, and a drifting steady state may not have been reached until A had undergone considerable fall. In the second place a drifting steady state may have been maintained throughout the phase of net synthesis, but there may have been a continuous change in the form of the steady-state P-A relation. If, for example, the proportions of the free amino-acids continuously approached those of the residues in the protein molecule, then it is possible that the total amino-nitrogen content could have fallen, while to maintain a steady state the protein content would have risen. The rate of such changes in proportions of individual amino-acids might be limited by the rate of formation of certain α-ketonic acids in respiration. That changes in the proportions of individual acids do occur has been seen in Fig. 5 with regard to asparagine and glutamine. The changes were not such as would account for the drift in the P-A relation, although it is conceivable that other changes occurred in the proportions of other amino-acids; but in any case it is not suggested that this was, in fact, the determinant concerned, since many other alterations may have taken place in the tissue. If proteins are formed by condensation of amino-acids, the rate of the forward reaction will depend on the amount of amino-acid at the seat of synthesis. The relation between this and the total amount in the cells may vary.

Characteristics of the Hydrolytic Phase.

The main factor causing the net hydrolysis of proteins was evidently the removal of amino-acids, which presumably followed concentration gradients set up between the fifth leaf and sinks in other parts of the plant; the P-A relationship governing the hydrolysis varied, however, according to past history.

It was suggested in previous papers (Watson and Petrie, 1940; Walkley, 1940) that this translocation factor might be the complete explanation of protein loss in leaves up to such time as the water content of the tissue began to fall; in this case there would be a single steady-state P-A curve with the course of which the system would tend to keep pace as the amino-acids are removed. If the form of the relation can be affected by treatment, however, it is also possible that the relation may drift with time; this would constitute the intrinsic factor of previous papers.

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[1] No evidence for such maxima was obtained in the experiments on total leaves described in the previous papers.
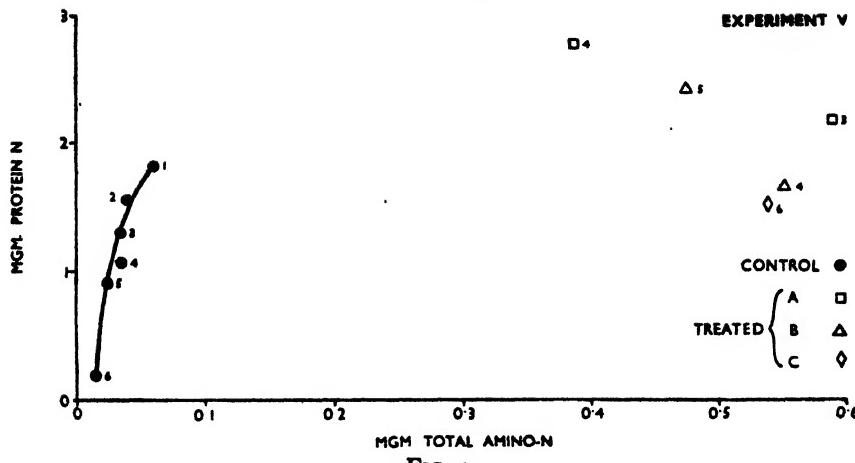
FIG. 4
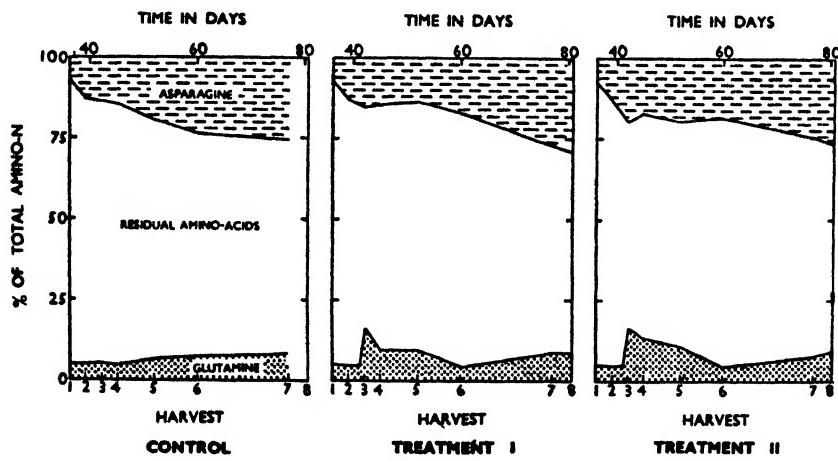
FIG. 5

FIGS. 4 and 5. Fig. 4. Absolute protein-nitrogen content plotted against absolute total amino-nitrogen content in the fourth leaf of barley. Ammonium sulphate treatments were given to three sets of plants, A, B, and C, on three successive occasions after maturity respectively. The harvest number is indicated for each point. For further experimental details see Walkley (1940). Fig. 5. Diagrams showing the proportions of the total amino-nitrogen in the fifth leaf of barley represented by glutamine-amino- and asparagine-amino-nitrogen. The treatment effects are insignificant except for the peaks in glutamine at harvests 3–5 ($P < 0.05$).

The lag in recovery of the P value when A was raised again by treatment conforms with this possibility. One explanation for the lag, as already suggested, might be that the P-A relation had drifted with time and only slowly reapproached its previous form; the data of experiment V (Fig. 4) suggest that the drift progressed with advancing loss of protein from the leaf. If P

continued to fall at the end of the experiment after A had become constant, this would also constitute strong evidence for a time-drift in the relation; unfortunately, however, the data are insufficiently sensitive to prove that the fall in A in reality ceased.

The present data thus point to the possibility of what was previously termed an intrinsic factor contributing to protein loss from the leaf, although they do not prove its existence. It should be added that there is no evidence that the change with time in the P-A relation would occur without the initiation of protein hydrolysis by fall in amino-acid concentration; the fact that restoration of A to its previous values more than restored the amount of protein present shows that there was no permanent shift in the system towards protein hydrolysis. The drifts in the proportions of the different amino-acids again indicate how the relation may have been affected by both time and treatment.

It may be concluded then that the P-A relation depends on the nutrient treatment of the plant, and also possibly on the protein content of the leaf, in such a way that, as the latter falls with age, the relation shifts in the direction of increasing hydrolysis.

SUMMARY

In a single mature leaf of the barley plant, the amounts of proteins and amino-acids decline with age, so that the relation between these two variables forms a curve concave to the amino-acid axis. If an additional supply of nitrogen is given to the plants while this drift is in progress, the values of the two variables are restored to high magnitudes, after which they again fall. The restoration in the case of the proteins, however, is comparatively slow, so that the decline in amino-acids sets in again before that of the proteins. The relation between the two variables during the declining phase is similar to that in the untreated plants but is not completely identical therewith, showing that the history of the leaf permanently affects the relation between proteins and amino-acids. It is possible also that the relation between these two variables changes with time—in other words, may be subject to an ontogenetic drift. ·

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